

FY 1993 ANNUAL REPORT

Biomedical Engineering and Instrumentation Program

National Center for Research Resources

National Institutes of Health

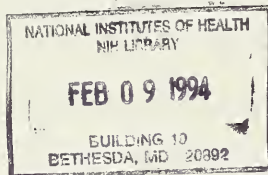




BIOMEDICAL ENGINEERING AND INSTRUMENTATION PROGRAM
NATIONAL CENTER FOR RESEARCH RESOURCES
NATIONAL INSTITUTES OF HEALTH

ANNUAL REPORT FOR FY 1993

Dr. Murray Eden, Director



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BEIP RESEARCH PROJECTS (Z01 RR)

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10001-25 BEI									
PERIOD COVERED October 1, 1992 to September 30, 1993											
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Pharmacokinetics											
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 40%;">Robert L. Dedrick, Ph.D.</td> <td style="width: 30%;">Chief</td> <td style="width: 30%;">CHES, BEIP, NCRR</td> </tr> <tr> <td>Cynthia Sung, Ph.D.</td> <td>Senior Staff Fellow</td> <td>CHES, BEIP, NCRR</td> </tr> <tr> <td>Paul F. Morrison, Ph.D.</td> <td>Physical Scientist</td> <td>CHES, BEIP, NCRR</td> </tr> </table>			Robert L. Dedrick, Ph.D.	Chief	CHES, BEIP, NCRR	Cynthia Sung, Ph.D.	Senior Staff Fellow	CHES, BEIP, NCRR	Paul F. Morrison, Ph.D.	Physical Scientist	CHES, BEIP, NCRR
Robert L. Dedrick, Ph.D.	Chief	CHES, BEIP, NCRR									
Cynthia Sung, Ph.D.	Senior Staff Fellow	CHES, BEIP, NCRR									
Paul F. Morrison, Ph.D.	Physical Scientist	CHES, BEIP, NCRR									
COOPERATING UNITS (if any) NTP, NIEHS; SNB, NINDS; University of Rochester School of Medicine; PB, NCI; MN, NINDS; Division of Clinical Pharmacology, FDA; LCS, NIMH											
LAB/BRANCH Biomedical Engineering and Instrumentation Program											
SECTION Chemical Engineering Section											
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, MD 20892											
TOTAL STAFF YEARS: 1.0	PROFESSIONAL: 1.0	OTHER: 0.0									
CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">_ (a) Human subjects</td> <td style="width: 33%;">_ (b) Human tissues</td> <td style="width: 33%;">X (c) Neither</td> </tr> <tr> <td>_ (a1) Minors</td> <td></td> <td></td> </tr> <tr> <td>_ (a2) Interviews</td> <td></td> <td></td> </tr> </table>			_ (a) Human subjects	_ (b) Human tissues	X (c) Neither	_ (a1) Minors			_ (a2) Interviews		
_ (a) Human subjects	_ (b) Human tissues	X (c) Neither									
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_ (a2) Interviews											
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Pharmacokinetic models are developed for the distribution and disposition of drugs, environmental contaminants, and endogenous metabolites in animals and humans. They provide a plausible set of equations that can be used to extrapolate data from animals to humans, and thereby improve chemotherapy and risk assessment. A pharmacokinetic model has been published for the pharmacokinetics of methyl mercury and inorganic mercury derived from it by demethylation in the growing rat. Preliminary consideration has been given to the pharmacokinetics of IBZM to include bound, free, and metabolite concentrations in relevant tissues. Work is well advanced on the development of a pharmacokinetic model for topotecan in the Rhesus monkey. Important features of the model include the reversible opening of the topotecan lactone to an hydroxy acid form, and transport between the plasma and the cerebrospinal fluid. Other research on regional therapy has included discussion of a draft clinical protocol for the administration of AZT into the cerebrospinal fluid for the treatment of AIDS dementia and related transport studies in the rat brain. A draft chapter on intraperitoneal drug administration has been prepared, and calculations suggest that absorption of drugs directly into the surface of the liver may be quantitatively more important than was previously recognized.											

RELEVANT BEIP PROJECTS: 73-159, 89-108, 89-116, 91-140, 93-103.

OBJECTIVES: To improve and extend mathematical models for the distribution and disposition of drugs, environmental contaminants, and endogenous metabolites in animals and humans to:

- (1) Account for species differences in drug distribution.
- (2) Provide a rational basis for extrapolating toxicity from experimental animals to humans.
- (3) Provide a basis for optimizing chemotherapy, with particular emphasis on regional drug delivery.
- (4) Enable rational transfer of *in vitro* thermodynamic and kinetic data to *in vivo* cases.

METHODS EMPLOYED: Mathematical models are developed from physicochemical, physiological, and anatomical information and the principles of chemical reaction engineering. Resulting ordinary or partial differential equations are solved analytically or numerically and compared with experimental data. Uncertainties are clarified by additional experiments and model modification.

(1) The increased emphasis that has been placed on the pharmacokinetics of macromolecules such as diphtheria toxin (DT), immunotoxins, and monoclonal antibodies (MAbs) has led to a separate IR Project Report on "Transport of Macromolecules" (C. Sung).

(2) We have published a physiological pharmacokinetic model for methyl mercury and its metabolite, mercuric mercury, in the growing rat. Demethylation appears to occur in both host tissues and gastrointestinal flora with elimination dominated by biliary secretion of inorganic mercury and by transport of methyl mercury into the gut lumen followed by substantial bacterial metabolism. Biliary transport of both organic and inorganic mercury is modeled in terms of the known secretion of glutathione from the hepatic pool. At 98 days following an oral tracer dose of ^{203}Hg -labeled methyl mercury chloride, 65% of the administered dose had been recovered in the feces as inorganic mercury and 15% as organic mercury. Urinary excretion is a minor elimination route, accounting for less than 4% of the dose as methyl mercury and 1% of the dose as inorganic mercury. Irreversible incorporation of the mercurials into hair is a significant route of elimination. Ten percent of the administered dose was contained in the hair shed during the 98 days and over 12% of the dose (almost 90% of the body burden) remained in the hair at the end of that time period. Apparent ingestion of hair by the rats during grooming represents a novel form of toxin recirculation. Transport of both chemical species between blood and tissues is bidirectional and symmetric with relatively slow movement into and out of the brain. Transport mechanisms for both mercurial species

are discussed in the context of capillary transport physiology and the blood-brain barrier to small molecules and proteins (Farris et al., 1993).

(3) Hair taken from rats dosed with ^{203}Hg -labeled methyl mercury was fed to previously untreated rats in order to determine if the organomercurial was available for release from the hair matrix within the gut lumen and for subsequent systemic absorption. Cumulative fecal excretion data were consistent with an absorption of about 80% of the ingested methyl mercury. The relative amounts of methyl mercury and of its metabolite, inorganic mercury, in the feces indicated that the percentage of the parent compound released from hair within the intestine equaled or exceeded the estimated bioavailability. Radioactivity in tissues of animals sacrificed 42 hr following hair consumption confirmed that mercury absorption had occurred (Farris and Dedrick, in press).

(4) For the treatment of leptomeningeal neoplasia, drugs can be administered directly into the cerebrospinal fluid (CSF). Clinical interest has been enhanced by the development of pharmacokinetic theory that predicts a large pharmacokinetic advantage in many cases. We are collaborating in studies to characterize the pharmacokinetics of topotecan, a topoisomerase I inhibitor and potential anticancer drug. Data from preclinical studies in monkeys receiving injections of the drug systemically or directly into the cerebrospinal fluid (CSF) are being analyzed with a physiologically based compartmental model. The data may be described by a model comprised of three compartments (CSF, central, and body). Features of the model include symmetrical transport across the blood-brain barrier, rapid exchange between plasma and body compartments, elimination of the intact and hydrolyzed forms of the drug at the same rate, and reversible hydrolysis of drug to an inactive form. The model may also prove useful for interpretation of clinical data on topotecan.

(5) A chapter has been drafted (with M. F. Flessner) on "Intraperitoneal Chemotherapy" for *A Textbook of Peritoneal Dialysis*, a new book in preparation, edited by R. Gokal and K. D. Nolph. In the course of our examination of the distributed model for peritoneal transport, it appeared that the surface of the liver may be much more important for the uptake of drugs than has previously been thought. Our theoretical calculations suggest that the liver could account for almost half of the clearance of sucrose from the peritoneal cavity of the human even though it has only about 13 percent of the surface area. This could help to account for the relatively small effect of evisceration on peritoneal transport in experimental animals. If correct, these calculations indicate that the amount of drug entering the liver through the portal vein is much less than frequently assumed. Only a small fraction of the metabolic capacity of the liver would be available to drugs absorbed directly into the liver so that partial saturation of metabolism could occur at a lower concentration than would be expected.

(6) Research has been initiated to determine if IBZM (S-3-¹²³I-iodo-N-[1-ethyl-2-pyrrolidinyl]-methyl-2-hydroxy-6-methoxybenzamide), used in conjunction with SPECT (single photon emission computed tomography), can be employed to determine D2 receptor densities in brain in a single scan. Raclopride, an analog of IBZM, is currently used to obtain such densities in PET (positron emission tomography), but the technique involves multiple exposure of patients to high radiation doses. Because the half-life of IBZM is longer than that of ¹¹-C-raclopride, the possibility exists that it can provide the same information in a single scan as opposed to the five used in PET. To accomplish this goal, a pharmacokinetic model of IBZM is being developed that describes bound, free, and metabolite concentrations in the brain, blood, lung, adipose tissue, muscle, and viscera. Because of its high lipophilicity, IBZM behaves much like the anesthetic thiopental except for large binding in the lung. Preliminary findings suggest that Scatchard-like plots can be obtained by sequential competitive removal by cold IBZM of hot IBZM bound to D2 receptors. This is more reliable than competitive removal by substances such as haloperidol since their use introduces (a) unknown and patient-specific parameters needed for data analysis and (b) the possibility of up-regulation of D2 receptors. It has also become clear that plasma concentrations need to be monitored on a continual basis. Present research is engaged in defining the minimal variables needed to be determined experimentally on each patient, and in identifying the optimal times for brain scans.

(7) Dementia is commonly associated with acquired immunodeficiency syndrome (AIDS). While the human immunodeficiency virus (HIV) does not appear to be neurotropic, cells such as microglia within the brain may be infected, and this can apparently lead to central nervous system pathology. Two small clinical studies have suggested that administration of AZT directly into the cerebrospinal fluid (CSF) is safe; one of these reported some therapeutic effect. We are continuing to collaborate in the design of, and in the development of the rationale for, a clinical trial. Calculations suggest a very large and possibly exploitable pharmacokinetic advantage associated with direct administration of AZT into ventricular CSF, but AZT removal from the CSF is relatively rapid; ventricular levels, therefore, are probably not representative of concentrations in the subarachnoid space. The depth of penetration of AZT into the brain from the CSF is a critical issue that we have examined in rats both experimentally and theoretically by means of microdialysis (see Z01 RR 10276-06 BEI).

SIGNIFICANCE: Drugs and other chemicals are tested for effects in animals and *in vitro* systems, with the aim of extrapolating the results to humans. Both the risk associated with environmental contaminants and the optimization of therapy are at issue.

PROPOSED COURSE: To continue pharmacokinetic modeling, with consideration of pharmacodynamic and cytokinetic events and drug interactions. To continue the support of regional procedures and

other measures to overcome drug resistance. To perform research designed to investigate distribution and metabolism of environmental contaminants, and to find methods for incorporating pharmacokinetics in models of risk assessment. To investigate chemical metabolism *in vitro*, in conjunction with pharmacokinetic models for quantitative prediction of metabolism *in vivo*. To extend distributed models for the description of drug movement through tissue.

PUBLICATIONS: Dedrick RL. Toxicology lessons from cancer chemotherapy. Chem Industry Inst Toxicol Activities 1992;12(7):1-7.

Morrison PF, Dedrick RL. Discovering switch pharmaceuticals from multistationary biochemical networks. [Editorial]. J Nat Cancer Inst 1993;85:518-9.

Flessner MF, Dedrick RL, Reynolds JC. Bidirectional peritoneal transport of immunoglobulins in rats: tissue concentration profiles. Am J Physiol 1992;263:F15-F23.

Farris FF, Dedrick RL, Allen PV, Smith JC. Physiological model for the pharmacokinetics of methyl mercury in the growing rat. Toxicol Appl Pharmacol 1993;119:74-90.

Farris FF, Dedrick RL. Absorption of methyl mercury from hair ingested by rats. Life Sci (in press).

leighton92formDEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10034-16 BEI
PERIOD COVERED October 1, 1992 to September 30, 1993		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Three-Dimensional Histological Reconstruction		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Stephen Leighton, Sc.D. Mechanical Engineer MES, BEIP, NCRR		
COOPERATING UNITS (if any) Marine Biological Laboratory (A. Kuzirian); NSS, NINDS (J. Olds)		
LAB/BRANCH Biomedical Engineering and Instrumentation Program		
SECTION Mechanical Engineering Section		
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, Maryland 20892		
TOTAL STAFF YEARS: 0.1	PROFESSIONAL: 0.1	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews ☐ (b) Human tissues ☒ (c) Neither		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) A semiautomatic system for acquiring three-dimensional structural information about histological material is being developed. The system should be faster and more reliable than techniques that use serial sections, although resolution may be limited. In brief, an embedded tissue block will be fixed relative to a scanning electron microscope imaging system; the surface of the block will be imaged and the image stored, and successive slices will be removed by a built-in microtome. Handling and registration of thin sections will thus be eliminated. Human and computer pattern recognition will transform the resulting set of images into a three-dimensional reconstruction. The images of <i>Hermisenda crassicornis</i> obtained by this technique correlate well with TEM images of the same tissue.		

OBJECTIVES: (1) To facilitate making schematic diagrams of neural networks. (2) To facilitate developmental studies of small organs and organisms. (3) To do three-dimensional reconstruction of biological structures.

METHODS EMPLOYED: In order to improve the resolution obtainable with our technique of Serial Block Face Imaging (SBFI), we plan to use frozen tissue cut with an ultracryomicrotome within the SEM, freeze-etched, and metallized. To further assess the feasibility of an ultracryomicrotome system within an SEM, a commercial cryochamber was attached to an SEM for preliminary experiments with cryopreserved tissue. Crude manual techniques were refined and the equipment was improved until preliminary results could be obtained. Water and ethanol were tested as embedding media.

MAJOR FINDINGS: Water, the obvious cryoembedding medium, appears to have acceptable properties for the cryoembedding, microtomy, etching, coating, and viewing that we wish to attempt. It has the additional advantage of a body of literature of comparable studies. The equipment must be further refined to achieve useful results.

PROPOSED COURSE: The cryochamber will be further improved to reduce contamination. Studies will be made to determine optimum process temperatures.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10039-16 BEI
PERIOD COVERED October 1, 1992 to September 30, 1993		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Biophysical Instrumentation and Methodology		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Marc S. Lewis, Ph.D. Research Chemist OD, BEIP, NCRR		
COOPERATING UNITS (if any) DCRT, PSL (R. Shrager); NCI, LB (S-J. Kim)		
LAB/BRANCH Biomedical Engineering and Instrumentation Program		
SECTION Microanalysis Section		
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, MD 20892		
TOTAL STAFF YEARS: 0.3	PROFESSIONAL: 0.3	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews ☐ (b) Human tissues ☒ (c) Neither		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The project is intended to develop new instrumentation and methods, and to improve existing instrumentation and techniques, for the characterization of biological macromolecules and the study of their interactions. Analytical ultracentrifugation, the techniques ancillary to it, and methods of data analysis using mathematical modeling appropriate for these techniques are the major areas of interest. Studies have continued on the application of mathematical modeling to problems of ultracentrifugal analysis, and on the development of new methods of performing experiments and analyzing data. In particular, a major breakthrough in the analysis of protein-nucleic acid and protein-protein interactions has been achieved by devising a new method of performing experiments and analyzing data.		

OBJECTIVES: To develop data acquisition systems for analytical ultracentrifuges; to develop appropriate software for use with these acquisition systems; to develop new methods of using new analytical ultracentrifuges; to develop new analytical methods applicable to these new experimental techniques, using MLAB software for the analysis of ultracentrifuge data by mathematical modeling techniques.

METHODS EMPLOYED: Mathematical modeling studies have involved the use of computer simulations for the development of the new technique, followed by the successful analysis of experimental data. The new experimental and analytical technique is applicable to any interacting system in which the two reactants have different absorption spectra. Typical examples are protein-nucleic acid interactions or protein-protein interactions in which one of the reactants has a chromophore modified to give a different spectrum.

MAJOR FINDINGS: This new method was designed for using data from the new Beckman XL-A analytical ultracentrifuge, and is dependent on the extended wavelength capabilities of this instrument. The technique involves obtaining absorbancy data at wavelengths in the range of 230 nm to 246 nm, as well as at 260 nm and 280 nm for the protein solution, the nucleic acid solution, and a solution of these reactants and their complex. The data from the protein and nucleic acid solutions permit the calculation of the molar extinction coefficients of these reactants as functions of wavelength. These calculations can be used to construct an extinction coefficient matrix that is a function of either protein or nucleic acid and wavelength. The data from the solution of reactants and complex permit the construction of an absorbancy matrix that is a function of radius and wavelength. If the pseudoinverse of the extinction coefficient matrix is multiplied by the absorbancy matrix, the resulting two-column matrix will consist of molar concentrations of protein plus complex and nucleic acid plus complex, respectively, as a function of radius. This matrix is then concatenated with a matrix of appropriate radial values, and the resultant data matrix can then be analyzed by mathematical modeling to obtain the value of the natural logarithm of the equilibrium constant. The computer simulations demonstrate that the generating value of $\ln K$ can be recovered with less than 0.1% error, in spite of the presence of substantial random error added to the absorbancy data, and that adding as much as 10% random error to the extinction coefficient results in less than a 2% error in the value of $\ln K$. Experimental studies have demonstrated that this method can be applied with equal facility to data obtained with the XLA ultracentrifuge. Computer simulations also indicate that this method can be used for the study of protein-protein interactions, provided that one of the proteins has 5-hydroxy tryptophan replacing normal tryptophan. This changes the absorption spectrum of the altered protein sufficiently that this method is applicable. Experimental verification is in progress.

SIGNIFICANCE: The combination of multiwavelength scans with this new method of data analysis provides the most robust and precise method yet developed for the ultracentrifugal analysis of protein-protein and protein-nucleic acid interaction, and offers optimum possibilities for thermodynamic analyses of interactions that are of critical importance in molecular biology.

PROPOSED COURSE: To continue studies of the type described above. A manuscript describing this work has been submitted for publication, and a book chapter describing it in greater detail has been completed.

PUBLICATIONS: Lewis MS. Data acquisition and analysis systems for the absorption optical system of the analytical ultracentrifuge. In: Harding SE, Rowe A, Horton JC, eds. Analytical ultracentrifugation in biochemistry and polymer science. Cambridge, U.K.: Royal Society of Chemistry, 1992;126-37.

Attri AK, Lewis MS. A fitting function for the analysis of sedimentation velocity concentration distributions. In: Harding SE, Rowe AJ, Horton JC, eds. Analytical ultracentrifugation in biochemistry and polymer science. Cambridge, U.K.: Royal Society of Chemistry, 1992;138-46.

Lewis MS, Shrager R, Kim S-J. Ultracentrifugal analysis of protein-nucleic acid interactions using multiwavelength scans. Colloid and Polymer Science 1993 (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10097-13 BEI
PERIOD COVERED October 1, 1992 to September 30, 1993		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Studies in Cardiovascular Dynamics		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Richard Chadwick, Ph.D. Head, Theor. Biomech. Group OD, BEIP, NCRR		
COOPERATING UNITS (if any) INSERM U. 120 (A. Azancot-Benisty); INSERM U. 141 (B. Levy); Laboratory of Physical Mechanics, University of Paris XII (J. Ohayon, C. Oddou); Pennsylvania State University (C. Dong)		
LAB/BRANCH Biomedical Engineering and Instrumentation Program		
SECTION Office of the Director		
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, MD 20892		
TOTAL STAFF YEARS: 0.3	PROFESSIONAL: 0.3	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) X ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) We have refined our existing model for myocardial and ventricular mechanics to include the effects of collagen on the elasticity of the myocardium and filling of the left ventricle; wall thickness and curvature effects; pathological fiber organization; myocardial blood flow; myocardial oxygen demand; effects of electrical activation patterns; interaction with the right ventricle; and dynamic vibrations. The model was used in conjunction with ultrasonic kinematic data, gated radionuclide ventriculography, and left-heart catheterization data; and implanted ultrasonic crystals were used to determine performance and contractility parameters. The physiological relationships between phasic coronary arterial, venous, and left ventricular pressure and blood flow were determined during a long diastole and under controlled conditions. A theoretical model was developed and was used to describe contraction of arterioles; it will be used to study mechanisms of hypertension. Theoretical models investigating residual stress in the myocardium and contraction with an ischemic zone are being developed. Congenital heart defects with right ventricular pressure and/or volume overload have been modeled.		

OBJECTIVES: To provide a framework for determining ventricular function, both at the fiber level and for the entire structure; to relate the relationship between coronary pressure and flow to the poroelastic rheology of the myocardium; to provide a framework for evaluating those aspects of cardiovascular function that depend on the filling of, and ejection from, the left ventricle; to develop new noninvasive measurement techniques for cardiovascular research; and to provide a theoretical framework for the prediction of myocardial ischemia and the severity of congenital heart defects.

METHODS EMPLOYED: An interdisciplinary approach is used involving clinical ultrasound data, hemodynamic measurements on animals, physical models, and mathematical models.

SIGNIFICANCE: This research program, with its interdisciplinary approach, has the potential to increase fundamental knowledge in cardiovascular physiology and to improve diagnosis and treatment of cardiovascular disease.

PROPOSED COURSE: Experiments are planned with INSERM U. 141 to investigate the effects of vessel stretch by volume loading on coronary blood flow. The theoretical model will be extended to include this effect. Experiments are also being planned to determine the mechanical properties of arterioles as a function of muscle tone and transmural pressure, and interpretation will be made with the theoretical model. The theoretical model of the left ventricle is being extended to deal with regional abnormalities. This extension necessitates developing mathematical techniques to deal with nonaxisymmetric conditions.

PUBLICATIONS: Chadwick RS, Dong C. Theoretical relationship among myocardial tissue and cavity pressures, contractility, and vascular volume change. In: Maruyama Y, Kajiyama F, Hoffman JIE, Spaan JAE, eds. Recent advances in the coronary circulation. Tokyo: Springer-Verlag, 1993 (in press).

Chadwick RS, Azancot-Benisty A, Ohayon J, Ito Y. Right and left ventricle interaction and remodeling in congenital heart disease. In: Sideman S, Beyar R, eds. Interactive phenomena in the cardiac system. Plenum Publishing Corporation, 1993 (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10098-13 BEI
PERIOD COVERED October 1, 1992 to September 30, 1993		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Laser Instrumentation for Vitreous and Cardiovascular Microsurgery		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation) Robert Bonner, Ph.D. Physicist EEES, BEIP, NCRR		
COOPERATING UNITS (if any) Clinical Branch, NEI (C. Kupfer); Optical Science Branch, Naval Research Laboratory; Cardiology Branch, NHLBI (S. Epstein); Quantronix Corporation; Spectraphos; Intratherapy, Inc.; Washington Cardiology Center		
LAB/BRANCH Biomedical Engineering and Instrumentation Program		
SECTION Electrical and Electronic Engineering Section		
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, MD 20892		
TOTAL STAFF YEARS: 0.2	PROFESSIONAL: 0.2	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) X ☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews ☐ (b) Human tissues ☐ (c) Neither		
SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.) We initially developed laser microsurgical techniques centered on ophthalmological applications (pulsed carbon dioxide lasers and pulsed ND:YAG slit lamp-based laser systems). Our primary focus for the last seven years, however, has been in cardiological applications--in particular, laser angioplasty and the development of pulsed, solid-state infrared lasers for microsurgery. We demonstrated the feasibility of transmission through flexible fiber optics of pulsed IR, visible and UV lasers, and tissue responses to these lasers. Specifically, we measured the dependence of acoustic transients and ablation efficiency on increasing pulse fluence with catheter systems used clinically. We concluded that acoustic transients play a dominant role in the clinical results of pulsed laser angioplasty. We developed new IR laser sources in conjunction with the Naval Research Lab and Quantronix Corporation that utilize strong water absorption and can be transmitted through low-loss, clad optical fibers. Clinical trials of peripheral and coronary laser angioplasty were completed, using either pulsed-dye or pulsed infrared lasers with computer-controlled fluorescence guidance. Recent work has involved identification of the tissue and biological effects of pulsed lasers--in particular, the large role that acoustic transients and transient, moderate (~60°C) thermal elevations play in acute and chronic clinical responses. Since pulsed laser angioplasty primarily disrupts the atheroma at stenoses mechanically rather than ablating or removing it, we must balance optimization of clinical results against increased rates of dissection.		

OBJECTIVES: Current objectives are: (1) to evaluate the relative advantages of various pulsed laser sources for angioplasty (pulsed-dye, excimer, and Tm:YAG lasers); (2) to design and develop microsurgical systems using these sources; (3) to determine the proper methodology for using these laser systems; (4) to evaluate the performance of these systems both *in vitro* and *in vivo*, and in comparison with new, alternative nonlaser techniques; and (5) to examine other areas of clinical importance when using these devices, in particular, the response of tissues to acoustic and thermal injury (e.g., restenosis).

METHODS EMPLOYED: Careful dosimetry of laser-tissue effects determines relative efficacy of the different laser sources. Tissue histological damage, as well as thermal transients surrounding the ablated volume, are quantified. Variation in laser pulse parameters (wavelength, temporal structure, and spatial structure) is performed in order to characterize the physical processes involved, and to understand and predict effects. A variety of physical detection methods have been used in order to evaluate effects and test models of laser damage in tissue, both *in vitro* and *in vivo*. Final evaluation of the laser system performance is based on intravascular ultrasound examination of clinical lesions before and after therapy in conjunction with long-term follow-up.

MAJOR FINDINGS: (1) Pulsed-dye laser angioplasty was able to cross total occlusions in patients, principally by the disruptive force of acoustic transients associated with tissue and blood vaporization. (2) Blood-enhanced local absorption on/within tissue allowed success of multifiber catheters at pulse fluences below the ablation threshold observed in the absence of blood. Fluorescence spectroscopy during laser sequences confirmed the presence of thin blood layers $>50\mu\text{m}$ between $\sim 70\%$ of the optical fibers and the tissue. (3) Associated with the large acoustic transients generated by rapid expansion of vapor bubbles was a high incidence of coronary artery dissections. (4) Laser-induced shock waves can pace ventricular contraction (though ECG-gating of laser pulses to the ventricular refractory period or high pulse repetition rates can inhibit the abnormal pacing). (5) Acoustic transients appear to tear the annuli of stiff plaque associated with stenoses and thereby overcome their constraint of vessel lumen, thereby allowing lumen expansion. However, this mode of action appears to be intrinsically prone to a high rate of dissection, and is only associated with minimal debulking of stenotic lesions. Pulsed laser angioplasty has been, in our studies and in others, generally unsuccessful in very stiff, highly calcified stenoses. (6) New "smooth-pulse" Tm:YAG (or Er:YAG) laser systems that we developed with NRL can ablate with smaller acoustic transients and may reduce the dissection rate, but their efficacy in angioplasty might also be limited.

These ambitious laser coronary angioplasty systems might provide possibilities for advancements in microsurgery in a variety of other fields.

SIGNIFICANCE: The successful development of an efficacious means of relieving atherosclerotic obstructions in human blood vessels (particularly the coronary arteries) by percutaneous laser angioplasty could have a dramatic effect on the practice of cardiology and vascular surgery, if the clinical results could be improved such that they become superior to those of balloon angioplasty or other, newer mechanical atherectomy devices. The Tm:YAG laser/silica optical fiber systems and Er:YAG laser/fluoride fiber that we have worked on appear to have numerous applications in other areas of endoscopic microsurgery.

PROPOSED COURSE: Refinement of current prototype laser microsurgery systems; testing of their applicability to laser angioplasty, ophthalmic microsurgery, and ablation of bone through *in vivo* and *in vitro* studies, with evaluation of efficacy in clinical trials.

PUBLICATIONS: Leon MB, Bonner RF. Future directions in laser angioplasty. In: Topol E, ed. *Interventional Cardiology*. 1993 (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10109-13 BEI									
PERIOD COVERED October 1, 1992 to September 30, 1993											
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Adjunctive Heat Treatment of Cancer											
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">Ronald L. Levin, Sc.D.</td> <td style="width: 33%;">Biomedical Engineer</td> <td style="width: 33%;">MES, BEIP, NCRR</td> </tr> <tr> <td>James Mattiello, Ph.D.</td> <td>Staff Fellow</td> <td>MES, BEIP, NCRR</td> </tr> <tr> <td>Ching-Nien Chen, Ph.D.</td> <td>Physical Scientist</td> <td>OD, BEIP, NCRR</td> </tr> </table>			Ronald L. Levin, Sc.D.	Biomedical Engineer	MES, BEIP, NCRR	James Mattiello, Ph.D.	Staff Fellow	MES, BEIP, NCRR	Ching-Nien Chen, Ph.D.	Physical Scientist	OD, BEIP, NCRR
Ronald L. Levin, Sc.D.	Biomedical Engineer	MES, BEIP, NCRR									
James Mattiello, Ph.D.	Staff Fellow	MES, BEIP, NCRR									
Ching-Nien Chen, Ph.D.	Physical Scientist	OD, BEIP, NCRR									
COOPERATING UNITS (if any) DR, CC (D. LeBihan); LCE, NHLBI (R. Turner)											
LAB/BRANCH Biomedical Engineering and Instrumentation Program											
SECTION Mechanical Engineering Section											
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, Maryland 20892											
TOTAL STAFF YEARS: 0.2	PROFESSIONAL: 0.2	OTHER: 0.0									
CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">X ☐ (a) Human subjects</td> <td style="width: 33%;">☐ (b) Human tissues</td> <td style="width: 33%;">☐ (c) Neither</td> </tr> <tr> <td>☐ (a1) Minors</td> <td></td> <td></td> </tr> <tr> <td>☐ (a2) Interviews</td> <td></td> <td></td> </tr> </table>			X ☐ (a) Human subjects	☐ (b) Human tissues	☐ (c) Neither	☐ (a1) Minors			☐ (a2) Interviews		
X ☐ (a) Human subjects	☐ (b) Human tissues	☐ (c) Neither									
☐ (a1) Minors											
☐ (a2) Interviews											
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The purpose of this project is to develop adjunctive hyperthermia modalities for cancer treatment through theoretical and experimental studies of the spatial and temporal variation in the temperature field of tissues subjected to radio-frequency electromagnetic radiation. We are currently refining a noninvasive method for measuring temperature in vivo, using magnetic resonance imaging of molecular diffusion.											

OBJECTIVES: To optimize the energy deposition patterns of hyperthermia applicators; to continue to develop a generalized mathematical model that will predict the spatial and temporal variation of the temperature field within a tissue or organ being heated; to continue to develop a noninvasive method for monitoring temperature during hyperthermia; and to develop optimal adjunctive hyperthermia modalities.

METHODS EMPLOYED: Experimental measurements of the resulting thermal profiles within phantoms and animals being heated by our hyperthermia applicators will be accomplished through the use of a dedicated PC/AT computing system, its associated data acquisition system, a BSD-200 thermal monitoring subsystem, a Luxtron 3000 thermal monitoring subsystem, and an OMEGA 4.7-T NMR unit.

MAJOR FINDINGS: Temperature images are obtained noninvasively, with high accuracy (0.2°C resolution, using 2-min. scans), using images of molecular diffusion. Temperature MR images agree very well with temperature measurements obtained from fiber-optic probes placed inside gel phantoms and animals. The temperature resolution was found to be 0.2°C , with a spatial resolution of 1 cm.

SIGNIFICANCE: At present, the heat treatment of cancerous cells shows considerable promise in the management of cancer when combined with conventional radiotherapy and chemotherapy; nevertheless, there still remain numerous important problems that must be resolved. Of paramount importance is the problem of generating and controlling uniform temperature fields within tissues. This study will therefore attempt to facilitate the development of optimum hyperthermia modalities by theoretically and experimentally studying the temperature fields within tissues during hyperthermia.

PROPOSED COURSE: Nonclinical *in vivo* work is continuing, using the 4.7-T Omega unit for studying the temperature dependence of diffusion during hyperthermia, and the GE 1.5-T Signa for development of real-time MRI temperature monitoring methods, using echo-planar imaging techniques.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10112-13 BEI
PERIOD COVERED October 1, 1992 to September 30, 1993		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Analysis of Microcirculatory Blood Flow by Laser Doppler Scattering		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Robert F. Bonner, Ph.D. Physicist EEES, BEIP, NCRR John Cole, MSEE Electrical Engineer EEES, BEIP, NCRR		
COOPERATING UNITS (if any) ROB, DCRT, NCI; LAP, DCRT; PSL, DCRT (R. Nossal); TSI, Inc., St. Paul, MN		
LAB/BRANCH Biomedical Engineering and Instrumentation Program		
SECTION Electrical and Electronic Engineering Section		
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, MD 20892		
TOTAL STAFF YEARS: 0.4	PROFESSIONAL: 0.4	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) X ☐ (a) Human ☐ (b) Human (c) Neither subjects tissues ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) We have developed a clinically useful method (the laser Doppler instrument and its theory of operation) for clinical measurements of microcirculatory blood flow, the density of flowing red blood cells (RBCs), and mean RBC velocity. Assistance has been given to the commercialization of this methodology and to its application to clinical research. Our clinical studies have been directed toward examining normal and abnormal microvascular dynamics through noninvasive clinical studies of skin and nasal mucosa, and toward intraoperative clinical studies of muscle and the CNS. Considerable theoretical work has been directed toward finding an adequate construct with which to interpret the physical measurements, and refining the accuracy of the microcirculatory measurements. We have discovered abnormal microcirculatory patterns and responses in the skin of patients with sickle-cell disease, hypertension, certain cardiac circulatory syndromes, diabetes, and skin cancer. The microcirculatory effects of therapy are monitored with this technique, affording a better understanding of the microcirculatory components of these diseases.		

OBJECTIVES: To develop our understanding of the human microcirculation in health and disease, and to refine the clinical utility of laser Doppler measurements of the microcirculation.

METHODS EMPLOYED: Application of the laser Doppler flow meters to various clinical protocols is based on the understanding of this technique and of the microcirculation of the targeted tissue. Because very little clinical work has heretofore been performed on microcirculatory dynamics, protocols are frequently designed to detect significant abnormalities and then to characterize them. Theoretical modeling of the signal and, to a limited degree, of the microcirculation facilitates the interpretation of the microcirculatory measurements.

MAJOR FINDINGS: (1) The blood volume and mean RBC velocity signals, as well as the blood flow signals obtained by the laser Doppler method developed at the NIH, provide reproducible and clinically useful measurements. (2) A light diffusion theory and empirical optical measurements have better defined the sample volume and the absolute calibration of the measurements for a variety of tissues. (3) Myogenic vasomotion in a variety of human tissue can easily be detected and quantified. This activity is dramatically increased in a variety of diseases and may play a critical role in microcirculatory adaptation to microvascular or rheological abnormalities. (4) Reliable measurements in the central nervous system (CNS) of patients undergoing surgery can be made with hand-held probes, and appear useful in analyzing the local microvascular response during AVM surgery. (5) Skin blood flow measurements can be used to quantify sympathetic innervation in patients with surgical sympathectomy and those with neuropathy. (6) The measurement of microcirculatory response to photodynamic therapy of tumors has been evaluated during and following therapy. (7) Studies of the peripheral microcirculation in diabetics have identified a functional loss of maximal microcirculatory dilation, which may have an important role in the morbidity associated with impairment and loss of limbs in long-term diabetics. (8) Studies of the radiation dose response variability of skin erythema in patients undergoing radiation therapy are under way to find a method of optimizing radiation therapy cumulative dosing in a diverse patient population.

PROPOSED COURSE: To pursue clinical protocols in order to establish and evaluate the contribution of microcirculatory abnormalities to a variety of diseases. To refine the technology in order to facilitate laser Doppler measurements in a variety of tissues and, in particular, those accessible via endoscopy. To continue to assist commercial manufacturers in the development of accurate, reliable, and useful instruments based upon this technology. To develop automatic algorithms for digitally sampling mean flow, volume, and velocity data during a clinical protocol in order to create a reliable data base while excluding

artifactual values obtained during motion and transient hemodynamic changes.

SIGNIFICANCE: The clinical research studies performed in this project have provided new means of quantifying the microvascular changes (in particular, microvascular dynamics) in a variety of diseases, such as sickle-cell disease, hypertension, scleroderma, diabetes, and allergy, as well as during and following surgical and nonsurgical therapies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10122-12 BEI
PERIOD COVERED October 1, 1992 to September 30, 1993		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Microcomputer Applications for the NIH Biotechnology Unit (Pilot Plant)		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Thomas R. Clem, Sr., BSEE Electronics Engineer EEES, BEIP, NCRR		
COOPERATING UNITS (if any) LCDB, NIDDK (Y. Shiloach)		
LAB/BRANCH Biomedical Engineering and Instrumentation Program		
SECTION Electrical and Electronic Engineering Section		
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, Maryland 20892		
TOTAL STAFF YEARS: 1.25	PROFESSIONAL: 1.0	OTHER: 0.25
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews ☐ (b) Human tissues ☒ (c) Neither		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) This project is concerned with satisfying the instrumentation needs of the NIH Biotechnology Unit (Pilot Plant) using modern equipment and low-cost desktop- or laptop-sized personal computers (PCs). PCs have been acquired and installed to supply a variety of services, including process control, data acquisition, data analysis, and administrative functions. The instrumentation and process control functions use commercial equipment as much as possible, and are interfaced using standard I/O connections, such as the IEEE-488 GPIB and the RS-232 Serial I/O ports, as well as direct digital and analog I/O components in the desktop computer. A standard data acquisition and control program has been written and is being adapted for each fermentor system in the Biotechnology Unit. By providing the same operator interface for each system, users need to learn only one command structure to be able to operate any system. Using these techniques allows changes in the parameters measured or controlled to be accomplished relatively quickly and easily. Utilizing the computational capabilities of the computer/controller allows initial selection of the operating parameters and dynamic alteration of these parameters as the process continues, thus allowing optimization of yields or detailed study of the process parameters. Adaptive control techniques have been applied to the process, and further increases in product yield are being realized.		

OBJECTIVE: To increase efficiency and productivity of the Biotechnology Unit, to expand the fermentation process capability, and to develop methods for researching the fermentation process through the expanded use of inexpensive desktop personal computers and more sophisticated control and data acquisition programs.

METHODS EMPLOYED: Desktop personal computers and interface electronics, both commercial and custom-designed, are connected to laboratory instruments and to fermentation vessels via the IEEE-488 GPIB, via the RS-232, or by direct connections. Interfacing techniques are kept as versatile as practical to allow system changes to be accomplished relatively easily. The control programs are written in the high-level language Pascal so that program changes can be made easily. All data are stored in a general form (ASCII) that most data analysis programs can access. A CRADA, "Development of an Adaptive Control System for the Fermentation Process" (number DK0009), was concluded this year. This collaboration has involved the extension of the data acquisition and control programs to include adaptive control, which provides much better control of selected process parameters, such as dissolved oxygen, with a resultant increase in product yield.

SIGNIFICANCE: Computer monitoring and control of the fermentation process provide several significant advantages over manual methods. By using the computer to make decisions based on what is occurring in the fermentation process, parameters can automatically be altered to increase yields of a product or to produce it in purer form. The computer can also perform some of the "housekeeping" tasks associated with running a fermentation process that would normally occupy an operator. Complete records of any run are saved on disk storage for later analysis. Computerized data acquisition and control allow growth rate and other parameters to be assessed under a variety of environmental conditions. The presence of a computer in the Biotechnology Unit office provides administrative support in the form of recordkeeping and text editing. This support has taken on new importance in the light of recent concern over safety and requirements for detailed logs of materials grown in the Pilot Plant.

PROPOSED COURSE: To expand the system to monitor and control additional parameters. (Larger fermentation vessels with more modern control systems, in the process of being acquired by the pilot plant, will be interfaced with our current systems.) To apply knowledge gained from previous work to new systems being assembled in support of the new Protein Expression Laboratory. To apply adaptive control capabilities to the other systems in the Biotechnology Unit and the Protein Expression Laboratory. To investigate the relative merits of other growth control techniques, such as maintaining dissolved oxygen level by feeding nutrient based on changes in measured DO₂ level.

PUBLICATIONS: Hsiao J, Ahluwalia M, Kaufman J, Clem T, Shiloach J. An adaptive control strategy for maintaining dissolved oxygen concentration in high density growth of recombinant *E. coli*. In: Biochemical engineering VII: cellular and reactor engineering. New York: New York Academy of Sciences, March, 1991;320-33.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10162-11 BEI
PERIOD COVERED October 1, 1992 to September 30, 1993		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Wound Healing: Biology and Rheology		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Thomas L. Talbot, M.S. Mechanical Engineer ACES, BEIP, NCRR		
COOPERATING UNITS (if any) SB, DCT, NCI (J. Norton, G. Salomon); ROB, DCT, NCI (W. De Graff)		
LAB/BRANCH Biomedical Engineering and Instrumentation Program		
SECTION Applied Clinical Engineering Section		
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, Maryland 20892		
TOTAL STAFF YEARS: 0.1	PROFESSIONAL: 0.1	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) _(a) Human _ (b) Human X (c) Neither subjects tissues _(a1) Minors (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Preliminary studies have been completed with swine models. These studies involved stamping an indelible grid (10 cm x 10 cm) on the skin of an anesthetized swine, excising a strip of the skin (1 cm x 10 cm) out of the grid area, and approximating the incision edges with silk sutures. Photographs of the grid were taken before excision, after excision, and after suturing. These photographs are being analyzed to determine the impressed strain on the wound closure, and eventually to relate this information to wound breaking strength (WBS). Studies based on an anesthetized rat model that relates biological and pharmacological interventions to WBS have been completed. Certain groups were treated pharmacologically during the wound-healing process. Significant decrease in WBS was observed in these groups, compared to control groups. Further studies will include the comparison of a tumor-bearing group to control groups.		

MAJOR FINDINGS: Anesthetized rats, treated with 8 mg/kg adriamycin prior to (or on the day of) wounding, demonstrated decreased wound breaking strength in incisional wounds at all intervals after wounding.

Decreased amounts of collagen and DNA, lower mitotic rates, and decreased cellularity were noted in wound chambers from rats treated in this manner. In both the incisional wound and wound chamber models, rats treated with adriamycin seven days after wounding showed a less dramatic healing impairment. No differences in collagen type were noted between chambers from the adriamycin-treated rats and those from untreated rats. The data suggest deficient quantities of platelet, macrophage, and lymphocyte factors; a decreased rate of collagen synthesis; and impaired collagen remodeling. Wound healing was examined in two wound healing models in control rats and animals bearing methylcholanthrene-induced sarcomas, in order to evaluate experimentally the effects of tumors on wound breaking strength. In a dorsal incisional wound model, a significant decrease in wound breaking strength was noted from 19 days after the implantation of tumor onward. The amount of the breaking strength deficit progressed with the size of the tumor. In a wound chamber model, hydroxyproline levels were measured as an index of collagen content, and 3 H-thymidine incorporation was related to DNA content in order to give an index of DNA synthesis. Histology and collagen types were also examined in the wound chamber model. The tumor-bearing state produced no significant change in any of the parameters studied in the wound chambers. The tumor-bearing state appears to inhibit wound healing in skin wounds but has no effect on healing in a model of a deeper wound. This difference in healing in the two wound models may be explained either by a crosslinking deficiency or by differential capacities for healing different types of wounds in tumor-bearing rats.

TGF-beta, which is now a readily available growth factor as a result of recombinant techniques, has been reported as a wound healing enhancement agent. A series of experiments were run that incorporated our present wound model, to investigate the effects of various concentrations of TGF-beta at the wound site. Paired dorsal incisions were filled with an absorbable collagen matrix, to which either 0.5 µg, 5 µg, or 50 µg of TGF-beta was added. Preliminary results indicate that TGF-beta does indeed affect wound healing, as assessed by wound breaking strength. However, depending on the dosage, the wound healing effect may be an increased or decreased resistance to wound bursting.

Similar experiments were performed using anesthetized guinea pigs as the experimental model, for straightforward comparison of results from previously published reports demonstrating either wound healing enhancement or deterioration.

PROPOSED COURSE: More experiments are under way that will focus primarily on determining the dosage of TGF-beta to produce optimal wound breaking strength.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10184-10 BEI
PERIOD COVERED October 1, 1992 to September 30, 1993		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Physical Chemistry of Biological Macromolecules		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation) Marc S. Lewis, Ph.D. Research Chemist OD, BEIP, NCRR		
COOPERATING UNITS (if any) Center for Molecular Science, University of Texas Medical Branch (S. Wison, W. Beard); LB, NCI (C. Vinson, D. Chatteraj, S-J. Kim); LCB, NIDCD (S. Yoo); DCBDC, NCI (E. Appella); LCBG, NIDDK (H. Pollard)		
LAB/BRANCH Biomedical Engineering and Instrumentation Program		
SECTION Microanalysis Section		
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, MD 20892		
TOTAL STAFF YEARS: 0.7	PROFESSIONAL: 0.7	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews ☒ (b) Human tissues ☐ (c) Neither		
SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.) The purpose of this project is to study the physical properties of a wide variety of biological macromolecules, with the goal of correlating these properties to the structure and function of the macromolecules. The emphasis is on the thermodynamics of the interactions of these macromolecules and on their molecular size and shape. Analytical ultracentrifugation and mathematical modeling are the principal research techniques used. Studies on HIV reverse transcriptase have been directed toward investigating the thermodynamics of the homogeneous association of the P66 subunit, and the heterogeneous association of P66 with the P51 subunit. Studies on chromogranin A have been directed toward determining the domain of this protein responsible for aggregation, and investigating the effects of pH and calcium ion concentration on the thermodynamics of the aggregative properties of a peptide encompassing this domain. Studies on CEB-63 and CEB-35, leucine-zipper peptides, have been directed toward determining the thermodynamic parameters characterizing their reversible aggregation. Studies on heat shock proteins and nucleic acids have been directed toward investigating the nature of their interactions. Studies on the interaction of HIV-1 RNA with nucleocapsid protein have been directed toward a more quantitative understanding of this interaction. Studies on the beta amyloid peptide have been directed toward understanding the aggregative properties of this peptide and its possible relationship to Alzheimer's disease.		

METHODS EMPLOYED: The macromolecules have been isolated and purified from appropriate sources or have been prepared synthetically by conventional methods. The associations have been studied by establishing equilibrium gradients in the analytical ultracentrifuge, then analyzing the resultant data by means of mathematical modeling techniques using PC MLAB.

MAJOR FINDINGS: HIV Reverse Transcriptase: HIV reverse transcriptase is an enzyme involved in the replication of HIV, and is composed of two subunits, P66 and P51, which form a heterodimer. The effect of site-directed mutagenesis on this dimerization has been studied, and it has been demonstrated that a leucine-repeat motif, seen in P66 but not in P51, appears to be critical to the heterodimer formation.

Chromagranin A: Chromagranin A, a major protein of the secretory vesicles of the adrenal medulla, is involved in both the storage of epinephrine and norepinephrine, and the storage and release of calcium in chromaffin cells. Recent studies have involved the determination of the domain of this protein involved in the calcium- and pH-dependent monomer-dimer and monomer-tetramer aggregation. A peptide has been isolated and studied that comprises approximately one-tenth of the intact molecule and has the same pH and calcium ion aggregation dependency as the whole molecule. The differences between the thermodynamic parameters characterizing its interactions and those of the whole molecule indicate the role of the remainder of the molecule in influencing the interactions.

CEB-63 and CEB-35: Studies of CEB-63, a model leucine-zipper peptide, show that its reversible monomer-dimer equilibrium is characterized by large negative changes in both enthalpy and entropy. The negative entropy change suggests hydrogen bonding or van der Waals forces as the most probable forces involved in the interaction. The enthalpy change also suggests that the dimer formation involves a significant coil-to-helix transition. CEB-35 is similar to CEB-63 in that it has the same leucine-zipper domain, but it lacks the domain that binds to DNA. The thermodynamic parameters characterizing the association of this peptide are similar to those characterizing the association of CEB-63 but greatly diminished in magnitude, thus demonstrating the importance of the DNA-binding domain to the dimer formation.

Heat Shock Proteins and DNA: These studies have been directed toward an understanding of the mechanisms of the interactions of heat shock proteins and DNA, both by studying intact proteins and DNA and by studying the interactions of more limited domains of both molecules and determining the effects of size and composition.

HIV-1 RNA-Nucleocapsid Peptide Interaction: The peptide is a synthetically prepared 55-amino acid portion of the major HIV-1 virion nucleocapsid, and contains the active RNA-binding domain.

The RNA is a 44-base pair double-stranded segment of the non-coding 5-primer end of HIV-1 RNA. The binding is site-specific on the RNA for a stem-and-loop structure. It was demonstrated that binding to the protein induced highly cooperative RNA dimer formation, and that this dimerization is RNA length-dependent, since a 19-base pair RNA segment did not undergo dimerization upon binding to the peptide.

Beta Amyloid Aggregation: It has been postulated that beta amyloid, a 4.3-KDa peptide, may be a cause of Alzheimer's disease. Beta amyloid calcium channels in bilayer membranes have been observed *in vitro*, and it has been suggested that this calcium channel formation may be involved in the disease. These studies have demonstrated that the peptide appears to be monomeric at a concentration of 5 micromolar, a concentration found in the cerebrospinal fluid that also induces the formation of amyloid calcium channels in bilayer membranes. This phenomenon would appear to refute the idea that aggregation must precede channel formation; it also suggests that the peptide must be very strongly bound to the membrane, thus inducing further aggregation of amyloid in the membrane. The studies have also demonstrated that beta amyloid is capable of undergoing a very great degree of aggregation, since aggregates of a molar mass in excess of 100,000,000 (as well as lesser aggregates) have been observed. Current studies are directed toward investigating the more limited aggregation that initiates the process.

SIGNIFICANCE: The significance of all of these studies is the same: They present information that is significant in correlating the physical properties of biological macromolecules to their structure and function. The work on HIV-1 reverse transcriptase and the studies of RNA-nucleocapsid protein associations are particularly important, because they present new information pertaining to the role of these molecules in HIV-1 replication. Similarly, the work on beta amyloid may be of critical importance in understanding Alzheimer's disease.

PROPOSED COURSE: It is expected that work on these molecules and others of similar interest will be continued.

PUBLICATIONS: Goel R, Beard WA, Kumar A, Casas-Finet JR, Strub M-P, Stahl SJ, Lewis MS, Bebenek K, Becerra SP, Kunkel TA, Wilson SH. Structure/function studies of HIV-1 reverse transcriptase: dimerization defective mutant L289K. *Biochemistry* 1993 (in press).

DasGupta S, Mukhopadhyay G, Papp PP, Lewis MS, Chattoraj DK. Mechanism by which heat shock proteins DnaJ and DnaK stimulate DNA binding activity of the P1 replication initiator RepA. *Journal of Molecular Biology* 1993 (in press).

Yoo SH, Lewis MS. Dimerization and tetramerization properties of the C-terminal region of chromagranin A: a thermodynamic analysis. Biochemistry 1993 (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10214-09 BEI									
PERIOD COVERED October 1, 1992 to September 30, 1993											
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Photodynamic Therapy											
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 40%;">Paul D. Smith, Ph.D.</td> <td style="width: 30%;">Physicist</td> <td style="width: 30%;">EES, BEIP, NCRR</td> </tr> <tr> <td>Walter S. Friauf, MEE</td> <td>Chief</td> <td>EES, BEIP, NCRR</td> </tr> </table>			Paul D. Smith, Ph.D.	Physicist	EES, BEIP, NCRR	Walter S. Friauf, MEE	Chief	EES, BEIP, NCRR			
Paul D. Smith, Ph.D.	Physicist	EES, BEIP, NCRR									
Walter S. Friauf, MEE	Chief	EES, BEIP, NCRR									
ROB, DCT, NCI (A. Russo, T. DeLaney); SB, DCT, NCI (H. Pass, W. Sindelar, M. Walther)											
LAB/BRANCH Biomedical Engineering and Instrumentation Program											
SECTION Electrical and Electronic Engineering Section											
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, Maryland 20892											
TOTAL STAFF YEARS: 1.0	PROFESSIONAL: 0.8	OTHER: 0.2									
CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">X ☐ (a) Human subjects</td> <td style="width: 33%;">☐ (b) Human tissues</td> <td style="width: 33%;">☐ (c) Neither</td> </tr> <tr> <td>☐ (a1) Minors</td> <td></td> <td></td> </tr> <tr> <td>☐ (a2) Interviews</td> <td></td> <td></td> </tr> </table>			X ☐ (a) Human subjects	☐ (b) Human tissues	☐ (c) Neither	☐ (a1) Minors			☐ (a2) Interviews		
X ☐ (a) Human subjects	☐ (b) Human tissues	☐ (c) Neither									
☐ (a1) Minors											
☐ (a2) Interviews											
SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.) Photodynamic therapy (PDT), which is the interaction of light with hematoporphyrin derivative (HPD or Photofrin II), is in clinical use in several areas of application. Currently, PDT is being used clinically for bronchial obstructions, carcinoma <i>in situ</i> of the bladder, and intraperitoneal and pleural tumors. Specially designed and constructed fiber-optic probes are modified on a case-by-case basis and calibrated in terms of irradiance delivered to the tissue. Calibrated custom photodiodes are placed at strategic locations within the cavity to provide a record of irradiance values (see project Z01 RR 10272-06). Dosimetry for PDT is complex, due to the variable irradiation levels within the tissue, the sequestering of HPD, and tissue oxygenation.											

OBJECTIVES: To evaluate PDT as a modality for treatment of selected diseases. To enhance the effectiveness of PDT as a clinical procedure by reducing the complications associated with its use. To characterize tissue, permitting theoretical analyses that predict the irradiation level at any point in the tissue distant from the point of light injection.

METHODS EMPLOYED: Two argon-ion-laser pumped liquid-dye lasers produce up to 11 W at 631 nm. 15 W can be delivered using the 514-nm line selected from the argon-ion output. All laser light is transmitted to the operating room by 600- μ fused silica fibers, to which sterile fiber-optic probes are attached. Custom and commercial fiber-optic probes were developed from 200- μ and 600- μ fused silica fibers.

For bronchial obstructions and bladder work, specially designed fiber-optic probes were constructed to produce a uniform distribution in a cylindrical or spherical profile surrounding the fiber. Bronchial obstructions were treated through a bronchoscope in a fully anesthetized patient, with irradiance levels in the tumor of 400 mW/cm, for a total delivered treatment of 250 J/cm. Tissue surrounding the tumor site was treated with 250 mW/cm² to a total of 100 J/cm². Follow-up bronchoscopy was performed 72 hours later to remove necrotic tissue.

Intraperitoneal and pleural therapy involve a wide range of tumor types. Light delivery is designed to treat all surfaces within these cavities, and similar techniques are used in both modalities. An initial surgical resection of tumor to a thickness not exceeding 5mm is performed, and all adhesions resulting from previous surgery are removed. Two methods of light delivery are used: For the abdominal and pleural walls and organs, a wand is used that consists of an inflated tracheotomy tube filled with 0.5% intralipid solution, into which the treatment fiber(s) are inserted; for the small bowel and mesentery, a surface illumination mode is used. The wand removes all directionality of the laser light, and prevents an excessively high local irradiance of tissue. Power levels at the fiber tip are 2 kW/cm², which can cause coagulation of the tissue and consequential damage of the fiber. If tissue breakdown product is present on the end of the fiber, the optical energy is absorbed at the tip with generation of heat. Photodiodes are sewn into various locations in the peritoneum and pleural cavity in order to monitor the light distribution. 0.2% intralipid is introduced into the cavity to help produce uniform illumination of the surfaces. 550-nm long-pass filters are used to cover the surgical lights to reduce the PDT effect from these lights during the extensive surgery required. Treatment levels have been increased intraperitoneally to an overall irradiance of 7.5 J/cm² using 514 nm light, followed by irradiance of selected sites of tumor involvement using 630 nm light up to a level of 15 J/cm². At the power levels delivered remotely from the laser by 600- μ fiber, treatment times typically last three hours.

Intrapleural therapy is performed exclusively using 630-nm light with irradiance levels increased to 35 J/cm². Typical laser treatment times are two hours.

In both pleural and intraperitoneal therapies, the treatment times are dictated by photodiode sensors sewn into the cavity to indicate when the desired level has been achieved. A PC-based computer provides both an on-line display of the irradiance levels achieved at seven strategic locations and a chronological, permanent record of the procedure on disk.

In the bladder, a spherically emitting fiber is used, which is positioned within the bladder (inflated to approximately 70% of full volume) with the aid of ultrasound imaging. Treatment times are typically 20 minutes long, producing a total dose of 2000 to 2500 J at 630-nm. A prototype instrument is used to position 400- μ spherical sensors against the bladder wall. These sensors are being evaluated as an on-line dosimetry technique.

MAJOR FINDINGS: The clinical response to the bronchial treatment is encouraging for both total occlusion and narrowing of the bronchus. In all cases, the follow-up bronchoscopy removed necrotic tumor tissue.

A variety of tumor types have been treated in the intraperitoneal therapy with escalation of delivered dose. Response to the therapy is assessed by clinical symptoms and by analysis of cytological washings. Phase I dose escalation studies in both modalities are complete, with the final irradiance levels indicated above.

Treatment times, even with two high-powered lasers, can reach four hours; tissue penetrance is limited to a maximum of 5 mm with 630 nm light, and a maximum of 1 mm with 514 nm light. The latter, even with higher power levels available and increased photodynamic effect due to more efficient absorption, can still lead to long treatment times if substantial blood contamination is present in the intralipid medium.

The photodiode monitoring system is now used routinely to control therapy. Prime advantages of this system are its ability to account for fluctuations in laser power and for reduced irradiance due to blood contamination in the intralipid medium. A modified version is being evaluated for bladder work.

SIGNIFICANCE: Photoradiation therapy has been shown to be effective in certain instances, to have potential in others, and in some cases (e.g., pulmonary metastatic disease) to provide a unique approach to the treatment of cancer. Future acceptance of PDT will depend on several factors, some of which are addressed here. A procedure for assessing the appropriate level of irradiation is essential in designing zones of treatment. In addition, identifying responsive diseases and requisite treatment modalities is of prime importance with PDT.

PROPOSED COURSE: We will continue human clinical trials (Phase II/III), and investigate animal models as necessary to explore new approaches. Light penetrance studies will be incorporated with singlet oxygen detection methods to define the levels necessary to cause the cytotoxic effects of PDT. Evaluation and interpretation of photodiode and other techniques for assessing light delivery will continue.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10225-09 BEI
PERIOD COVERED October 1, 1992 to September 30, 1993		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Processing of High-Resolution Electron Micrographs		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Akram Aldroubi, Ph.D. Staff Fellow OD, BEIP, NCRR Michael Unser, Ph.D. Visiting Scientist OD, BEIP, NCRR		
COOPERATING UNITS (if any) CSL, DCRT (B. Trus); LPB, NIAMS (A. Steven)		
LAB/BRANCH Biomedical Engineering and Instrumentation Program		
SECTION Office of the Director		
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, MD 20892		
TOTAL STAFF YEARS: 0.5	PROFESSIONAL: 0.5	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) _ (a) Human _ (b) Human X (c) Neither subjects tissues _ (a1) Minors _ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Over the years, we have developed several computational techniques for the statistical analysis of sets of electron micrographs of biological macromolecules. These methods include various types of factorial analysis (correspondence analysis, principal components), outlier detection schemes, statistical criteria for the quantitative assessment of spatial resolution (spectral signal-to-noise ratios), and a fast algorithm for the determination of the scaling factors between two micrographs. A current goal is to be able to combine a larger number of micrographs with slight disparities in magnification, in order to obtain higher-resolution 3-D reconstructions of icosahedral viruses. For this purpose, we are developing new spline-based algorithms for various geometric corrections (arbitrary scaling, translation, and rotation). These methods are based on a general least squares principle.		

OBJECTIVES: There are currently four main objectives in this research: (1) The determination of appropriate statistical models (noise and various distortions) for high-resolution micrographs; (2) the study of algorithms for the registration of specimen views, using simple (translation/rotation) or more complex geometrical transformations; (3) the decision procedure that determines the classification of different views, as well as the detection of "bad" samples; and (4) the assessment of the quality of results obtained by averaging (e.g., signal-to-noise improvement, resolution). In particular, we have considered the problem of correcting for magnification mismatches between micrographs. Each image displays a series of independent projective views of the object under consideration. These views have different orientations and are not registered. Moreover, the micrographs may have different contrast.

METHODS EMPLOYED: In our formulation, the original and rescaled images are both represented by an interpolating spline with step size 1 and Δ , respectively. The change of scale is achieved by determining the spline with step size Δ that provides the closest approximation of the original signal in the L_2 -norm. This approximation is computed in three steps: (1) a digital prefilter, which provides the B-spline coefficients of the input signal; (2) a resampling, using an expansion formula with a modified sampling kernel that depends explicitly on Δ ; and (3) a digital postfilter, which maps the result back into the signal domain.

MAJOR FINDINGS: Our least squares resampling algorithm was found to compare very favorably with standard interpolation methods. It consistently produced images that are more faithful to the original, including lesser visual distortions and improved suppression of aliasing artifacts.

SIGNIFICANCE: The scientific expertise we have acquired and the software we have developed should allow other scientists to use these image processing and computer reconstruction techniques to determine or understand a specimen's structure. The derived methods and the software we developed constitute an important step in obtaining higher-resolution 3-D reconstruction of macromolecules.

PROPOSED COURSE: We will extend the present resampling approach so that it can also properly handle image rotations. Another problem is to correct for the effect of the contrast transfer function when combining several micrographs.

PUBLICATIONS: Aldroubi A, Trus BL, Unser M, Booy FP, Steven AC. Magnification mismatches between micrographs: corrective procedures and implications for structural analysis. Ultramicroscopy 1992;46:175-88.

Trus BL, Unser M, Pun T, Steven AC. Digital image processing of electron micrographs: the PIC system II. Scanning microscopy (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10256-07 BEI
PERIOD COVERED October 1, 1992 to September 30, 1993		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Mechanical Prosthetic Heart-Valve Tester		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Thomas L. Talbot, M.S. Mechanical Engineer ACES, BEIP, NCRR		
COOPERATING UNITS (if any) FDA (S. Stewart); NHLBI		
LAB/BRANCH Biomedical Engineering and Instrumentation Program		
SECTION Applied Clinical Engineering Section		
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, Maryland 20892		
TOTAL STAFF YEARS: 0.1	PROFESSIONAL: 0.1	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews ☐ (b) Human tissues ☒ (c) Neither		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Six different manufacturers' prosthetic heart valves were tested and ranked according to performance indices described by Swanson and Gabbay. Additionally, a technique was devised whereby the pressure drop across the aortic valve could be predicted by velocity measurements made with continuous-wave Doppler (CWD) imaging. The results of preliminary <i>in vitro</i> studies demonstrate a high correlation ($r > 0.9$) between the peak velocity as measured with CWD and the predicted velocity obtained by the application of a simplified Bernoulli equation, which uses a measured maximum pressure drop across the aortic valve. Also, an acoustical signature analysis technique is under investigation for possible noninvasive detection of partially failed single-leaflet-type mechanical valves.		

PROPOSED COURSE: The system works well with mechanical prosthetic valves, and the data acquisition and analysis portions of the project have been refined considerably. Plans include the modification of the system to facilitate the testing of explanted mechanical and bioprosthetic heart valves. Additional analysis has revealed errors in measuring prosthetic valve pressure gradients by continuous-wave Doppler, compared to standard catheter measurements. These errors appear systematic, and vary depending on the type of prosthetic valve. A manuscript detailing these observations and their clinical implications is in preparation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10257-07 BEI									
PERIOD COVERED October 1, 1992 to September 30, 1993											
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Analysis of Propagation of Light in Turbid Biological Tissues											
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 40%;">Robert F. Bonner, Ph.D.</td> <td style="width: 30%;">Physicist</td> <td style="width: 30%;">EEES, BEIP, NCRR</td> </tr> <tr> <td>Joseph M. Schmitt, Ph.D.</td> <td>Staff Fellow</td> <td>ACES, BEIP, NCRR</td> </tr> <tr> <td>Michael Yadlowsky, Ph.D.</td> <td>NRC Fellow</td> <td>EEES, BEIP, NCRR</td> </tr> </table>			Robert F. Bonner, Ph.D.	Physicist	EEES, BEIP, NCRR	Joseph M. Schmitt, Ph.D.	Staff Fellow	ACES, BEIP, NCRR	Michael Yadlowsky, Ph.D.	NRC Fellow	EEES, BEIP, NCRR
Robert F. Bonner, Ph.D.	Physicist	EEES, BEIP, NCRR									
Joseph M. Schmitt, Ph.D.	Staff Fellow	ACES, BEIP, NCRR									
Michael Yadlowsky, Ph.D.	NRC Fellow	EEES, BEIP, NCRR									
COOPERATING UNITS (if any) PSL, DCRT (R. Nossal, G. Weiss, A. Gandjbakhche); Department of Physics, Bar-Ilan University, Israel											
LAB/BRANCH Biomedical Engineering and Instrumentation Program											
SECTION Electrical and Electronic Engineering Section											
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, MD 20892											
TOTAL STAFF YEARS: 2.0	PROFESSIONAL: 2.0	OTHER: 0.0									
CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">_ (a) Human subjects</td> <td style="width: 33%;">_ (b) Human tissues</td> <td style="width: 33%;">X (c) Neither</td> </tr> <tr> <td>_ (a1) Minors</td> <td></td> <td></td> </tr> <tr> <td>_ (a2) Interviews</td> <td></td> <td></td> </tr> </table>			_ (a) Human subjects	_ (b) Human tissues	X (c) Neither	_ (a1) Minors			_ (a2) Interviews		
_ (a) Human subjects	_ (b) Human tissues	X (c) Neither									
_ (a1) Minors											
_ (a2) Interviews											
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.) Many of the clinical research projects of the laser and modern optics group involve the interaction of light with tissue (e.g., laser microsurgery, laser Doppler blood flow measurements, photodynamic therapy of cancer, noninvasive platelet assessment, and optical tissue oximetry). In order to quantitate these techniques more fully, we have undertaken theoretical modeling of light propagation in biological tissues and turbid media. Analytical equations have been devised to characterize various parameters of photons illuminating a tissue surface (including probability of surface re-emission at a given distance, mean path before re-emission, mean depth of penetration, and probability of absorption with depth). These expressions have been used to interpret empirical measurements on living tissues and to quantify a variety of clinical measurements (e.g., laser Doppler blood flow and volume measurements, dosimetry in PDT of cancer, and remote sensing of atherosclerotic plaque). Recently our theoretical predictions of pathlength distributions of re-emitted photons have been applied to temporal dispersion of picosecond laser pulses in brain and muscle. Such analysis may allow noninvasive, absolute quantitation of hemoglobin and myoglobin oxygen saturation <i>in vivo</i>, as well as the concentration of a variety of important biomolecules.											

OBJECTIVES: To develop an insight into light propagation in turbid homogeneous and layered biological tissues, and to provide analytical expressions of general utility.

METHOD EMPLOYED: Mathematical theory of random walks on lattices and both Monte Carlo and exact enumeration computer modeling have been used to develop a quantitative analysis of light propagation in biological tissues. Empirical measurements have been obtained from tissues using laser fiber optics systems. Comparison with diffusion theory has been examined.

MAJOR FINDING: A set of analytical equations of general usefulness when illuminating a tissue surface have been developed in reflection and transmission. New scaling relationships for light propagation with different anisotropy of scattering (i.e., similarity relations in analytical expressions for $\langle \cos \theta \rangle$, u_s , and u_a) have been quantified. Simple relationships have been derived for resolution of time-gated transillumination imaging of deeply embedded objects, and the tradeoff between signal intensity and resolution has been evaluated for the case of embedded optical abnormalities in the human female breast.

SIGNIFICANCE: Light is frequently used to probe and treat biological tissues; however, accurate evaluation of light propagation in tissue has been an arcane field yielding few clinically useful insights or relationships. As a result, most interpretation has been either qualitative or has required extensive correlations with techniques that have poorly-controlled optical variations. Our analytical theory gives readily applied equations for a variety of optical parameters of photon propagation in tissue.

PUBLICATIONS: Gandjbakhche AH, Nossal R, Bonner RF. Scaling relationships for theories of anisotropic random walks applied to tissue optics. *Applied Optics* 1993;32:504-16.

Gandjbakhche AH, Weiss GH, Bonner RF, Nossal R. Photon pathlength distributions for transmission through highly scattering slabs. *Physical Review Series E* 1993;48 (in press).

Schmitt JM, Knüttel A, Gandjbakhche AH, Bonner RF. Optical characterization of dense tissue using low-coherence interferometry. In: *SPIE Proceedings*. SPIE, 1993 (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10258-07 BEI
PERIOD COVERED October 1, 1992 to September 30, 1993		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Photochemical Inactivation of Virus and Bacteria in Blood		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Robert F. Bonner, Ph.D. Physicist EEES, BEIP, NCRR		
COOPERATING UNITS (if any) DBBP, FDA (J. Fratantoni); ROB, NCI		
LAB/BRANCH Biomedical Engineering and Instrumentation Program		
SECTION Electrical and Electronic Engineering Section		
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, MD 20892		
TOTAL STAFF YEARS: 0.1	PROFESSIONAL: 0.1	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews ☐ (b) Human tissues ☒ (c) Neither		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Photochemical sterilization of blood products is the ultimate goal of this project, in which we are quantitating photochemical destruction of virus in blood and blood products and the effects of the process on the native blood components. Initial studies using an XeCl excimer laser characterized the response of virus and blood components as functions of cumulative fluence and peak irradiance. Single- and multiple-photon photochemical schemes were investigated. Single-photon photochemistry resulted in an efficacious treatment range between 10 and 20 J/sq cm, in which a hardy virus is inactivated and platelets and plasma proteins are minimally affected. Multiple-photon effects at higher irradiance led to increased protein damage in blood components, without increasing efficacy of viral inactivation. Augmentation of this therapeutic window by photochemistry (using exogenous agents such as riboflavin, tetracycline, and hemocyanin) was investigated, with a focus on the adverse effects on platelets and the relative susceptibility of different viruses, depending on the complexity of their coats. The inhibitory effect of serum albumin on photochemistry was also examined. Proposed work will examine exogenous agents that show strong affinity for binding to DNA, and thereby might increase therapeutic efficacy. The high sensitivity of blood platelets to activation and/or damage by photochemical products (e.g., singlet oxygen) has led to a renewed emphasis on optical and immunological methods of monitoring minimal lesions associated with viral inactivation photochemistry, as well as standard blood-banking techniques of preparation and storage.		

OBJECTIVES: To develop an efficacious means to inactivate viral and bacterial agents in blood.

METHOD EMPLOYED: Photochemical treatment, using UV-laser effects on DNA or visible light sources interacting with exogenous photochemicals. Quantitative dosimetry and assays of viral inactivation and blood component function were used to determine dose response curves. Noninvasive optical assays utilizing low-angle multiple scattering of flow-oriented platelet suspensions allowed *in vitro* determination of functional changes in platelets.

MAJOR FINDINGS: UV-laser photochemical inactivation of virus has a therapeutic window of efficacy (10 to 20 J/cm² @ 308nm and ≤ 0.2 MW/cm²) in which platelet and plasma protein function are maintained. Two-photon photochemistry at higher irradiance (~ 1.4 MW/cm²) results in little improvement in viral inactivation but dramatic loss in blood component function, presumably due to peptide bond breakage. Sensitivity of blood cells (e.g., platelets) and the strong binding and/or quenching by serum albumin have proven to be severe limitations of the use of exogenous agents exhibiting non-DNA-specific photochemistry. Viral inactivation is also very sensitive to viral coat composition and its permeability to the exogenous agents.

SIGNIFICANCE: Transfusion risks associated with the transmission of infectious agents remain a major problem in transfusion medicine. A simple, effective, noninvasive method to inactivate these infectious agents would be of enormous clinical importance. We can envision possible patient treatment procedures using photochemical treatment of a patient's blood during plasma-pheresis.

PROPOSED COURSE: Experiments with psoralen photochemistry *in vivo* will explore complex intravascular bioeffects. A new emphasis will be the optimization of the use of the blood supply, which minimizes the number of donors to which a typical patient is exposed, thereby reducing cumulative risks. A comparative study, in which new immunological methods of sensing subtle changes in platelet membrane function will be correlated with noninvasive optical assessment, seeks to elucidate and refine rapid, routine noninvasive optical analysis of blood products.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10259-07 BEI
PERIOD COVERED October 1, 1992 to September 30, 1993		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Visual Target-Tracking Ability Assessment System		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Thomas R. Clem, Sr., BSEE Electronic Engineer EEES, BEIP, NCRR		
COOPERATING UNITS (if any) LPP, CNSB, NIMH (R. Litman); LSES, NIMH (C. Schooler, B. Roberts)		
LAB/BRANCH Biomedical Engineering and Instrumentation Program		
SECTION Electrical and Electronic Engineering Section		
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, Maryland 20892		
TOTAL STAFF YEARS: 1.0	PROFESSIONAL: 0.9	OTHER: 0.1
CHECK APPROPRIATE BOX(ES) X (a) Human (b) Human (c) Neither subjects tissues (a1) Minors (a2) Interviews		
SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.) Abnormal eye movements found in patients with central nervous system (CNS) diseases can sometimes be revealing about the underlying pathology. Although the oculomotor nuclei, their descending primary motor neurons, and the extraocular muscles ultimately mediate eye movements, higher centers (including premotor areas in the brain stem, midbrain, subcortical areas, and cerebral cortex) are also involved in the movements. The oculomotor nuclei and neurons, together with the higher motor centers, functionally comprise five oculomotor systems responsible for different types of eye movements. Of these, the smooth-pursuit eye-movement system and the saccadic eye-movement system are of interest, for they seem to be abnormal in CNS disorders involving higher subcortical and cortical centers. Moreover, both smooth-pursuit and saccadic eye movements have distinct and easily measured properties. Disorders of smooth-pursuit eye movements (SPEM) have been found in schizophrenia, CNS illnesses affecting the oculomotor nuclei (e.g., brainstem infarction), diffuse brain diseases (such as disorders of the Alzheimer's type), Huntington's disease, and Parkinson's disease.		

OBJECTIVE: To study the ability of subjects to follow a variety of target movements, to compare the results of normal subjects with those of subjects known to have dementia, and to define a set of parameters that will characterize abnormal conditions. A portable system has been fabricated that allows researchers to study patients and their family members in their homes or at a medical facility remote to the NIH. Tests are also being given to AIDS patients to assess CNS effects of HIV.

METHODS EMPLOYED: A custom interface was built to interconnect a commercial eye-position detector and a desktop PC. A second monitor connected to the PC provides the moving target, a small spot moved horizontally in a variety of patterns. Custom programs were written to produce the desired moving-target pattern and to collect the eye-position data. These programs allow graphic display of the data and some rudimentary analysis.

SIGNIFICANCE: This system allows researchers to acquire data that were unobtainable prior to its development. The use of the PC provides the advantages of flexibility of experimental parameters, speed and accuracy of data acquisition, simultaneous acquisition of data from several sources, and storage of raw data.

PROPOSED COURSE: As further data are acquired, new target challenges will be tried. We intend to continue this program of refinement and to alter our procedures as the results of our experiments direct. The system is being upgraded with a newer, faster computer. Improvements of data analysis and data reporting are being developed. Further tests and equipment are being developed to test a subject's abilities to coordinate eye movements with hand responses and auditory stimuli. Programs will be enhanced to allow multiple-target presentations, including moving distractor images (while maintaining the 1-millisecond sample rate of eye-position data acquisition), and to produce multiple-color displays using techniques to modify individual pixel attributes on the display. The electronic interface electronics will be modified to allow the use of the system with VGA video systems.

OBJECTIVES: To develop a greatly improved confocal laser microscope with no moving parts, in which the performance will exceed that of existing confocal microscopes in that it will generate images at video rates that are confocal in both lateral dimensions. Digital electronic chirp generation will enable wavelength changes in a simplified acousto-optic scanning system.

SIGNIFICANCE: The superior optical performance of the confocal microscope is now widely recognized in the biomedical community. Most commercially available systems use galvanometer laser scanners to generate the picture and are therefore slow, typically yielding images at an order of magnitude less than video rates. Commercial systems operating at video rates are confocal only in one, rather than in two, dimensions (with attendant disadvantages). Our system operates at TV-frame rates (partial fields operate at faster speed) and is confocal in both dimensions. The rapid operating speed should enhance ease of use and allow studies of rapidly occurring biological phenomena. The lack of moving parts is anticipated to result in greatly reduced maintenance and alignment problems, compared to existing units. Use of acousto-optic scanners to achieve the high scanning rates normally precludes changing the illumination wavelength easily. The new system, however, will overcome this problem electronically to give the microscopist a more flexible system that is more likely to be commercially accepted.

METHODS EMPLOYED: A very precise and repeatable raster scan of a laser spot is performed with two acousto-optic beam deflectors under digital control. A novel means of effectively scanning a pinhole in synchrony and alignment with the laser beam, using an image dissector tube, has been developed and patented. Light getting through the pinhole is sensed and used to modulate a raster scan on a TV monitor to generate the confocal image. The increase in speed is achieved by scanning the laser beam rather than mechanically scanning the object through a stationary laser beam. A personal computer controls all alignment procedures, as well as image generation. The system has no moving mechanical parts, except for the axial focus. A new laser scanner has been completed which uses Bragg deflectors with large circular entrance pupils to eliminate the need for anamorphic beam shaping optics. The resulting simplified optic system, combined with the capability to generate different chirps digitally, allows wavelength to be altered easily.

PROPOSED COURSE: The improved laser scanning system will be installed and tested. Uses for both reflected light and fluorescence are being evaluated.

PUBLICATION: Goldstein S, Hubin T, Smith T. An improved no-moving-parts video-rate confocal microscope. *Micron and Microscopica Acta* 1992;23:437-46.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10272-06 BEI												
PERIOD COVERED October 1, 1992 to September 30, 1993														
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Photometry for Photodynamic Therapy														
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">Walter S. Friauf, MEE</td> <td style="width: 33%;">Section Chief</td> <td style="width: 33%;">EEES, BEIP, NCRR</td> </tr> <tr> <td>John Cole, MSEE</td> <td>Electronic Engineer</td> <td>EEES, BEIP, NCRR</td> </tr> <tr> <td>Paul D. Smith, Ph.D.</td> <td>Physicist</td> <td>EEES, BEIP, NCRR</td> </tr> <tr> <td>Robert F. Bonner, Ph.D.</td> <td>Biophysicist</td> <td>EEES, BEIP, NCRR</td> </tr> </table>			Walter S. Friauf, MEE	Section Chief	EEES, BEIP, NCRR	John Cole, MSEE	Electronic Engineer	EEES, BEIP, NCRR	Paul D. Smith, Ph.D.	Physicist	EEES, BEIP, NCRR	Robert F. Bonner, Ph.D.	Biophysicist	EEES, BEIP, NCRR
Walter S. Friauf, MEE	Section Chief	EEES, BEIP, NCRR												
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Paul D. Smith, Ph.D.	Physicist	EEES, BEIP, NCRR												
Robert F. Bonner, Ph.D.	Biophysicist	EEES, BEIP, NCRR												
COOPERATING UNITS (if any) ROB, DCT, NCI (A. Russo, T. Delaney); SB, DCT, NCI (H. Pass)														
LAB/BRANCH Biomedical Engineering and Instrumentation Program														
SECTION Electrical and Electronic Engineering Section														
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, Maryland 20892														
TOTAL STAFF YEARS: 0.4	PROFESSIONAL: 0.4	OTHER: 0.0												
CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">X ☐ (a) Human subjects</td> <td style="width: 33%;">☐ (b) Human tissues</td> <td style="width: 33%;">☐ (c) Neither</td> </tr> <tr> <td>☐ (a1) Minors</td> <td>☐ (a2) Interviews</td> <td></td> </tr> </table>			X ☐ (a) Human subjects	☐ (b) Human tissues	☐ (c) Neither	☐ (a1) Minors	☐ (a2) Interviews							
X ☐ (a) Human subjects	☐ (b) Human tissues	☐ (c) Neither												
☐ (a1) Minors	☐ (a2) Interviews													
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Dosimetry for photodynamic therapy (PDT) involves measurement of hematoporphyrin derivative (HPD) concentration, oxygen concentration, singlet oxygen concentration, and therapeutic light level, all as a function of position throughout the tissue being treated (either intentionally or unavoidably). Noninvasive measurements are essentially limited to surfaces, although theoretical and empirical models can be used to estimate values some distance into the tissue. Fiber-optic probes can be used for direct subsurface measurements.														

OBJECTIVE: To quantitate principal parameters involved in PDT in experimental models, animal trials, and human treatment.

METHODS EMPLOYED: A small handheld probe was developed to measure surface concentration of HPD. The excitation is blue light from a coherent model PRT-100 laser, electromechanically chopped at 5 hz and conveyed to the tip of the probe by an optical fiber. The tip is conical, with an aperture 2 mm in diameter, allowing good spatial localization. Fluorescence of the HPD is separated from reflected excitation and tissue fluorescence by two filters, and sensed by a solid-state photodiode. The component at the chopped frequency is amplified and read by a digital voltmeter or, for very low-level measurements, by a lock-in amplifier. During the past year, additional experimental usage and verification have been carried out and use for other types of measurements has been explored.

For measurement of light delivered during intraperitoneal treatments, a twelve-channel, computer-based light monitoring system has been designed and built. Completely insulated solid-state photodiodes connected by coaxial cables are sutured into positions of interest within the abdominal cavity. The computer applies calibration constants for each diode and integrates the light dose at each site in real time. Multiple isolation transformers and special construction features insure freedom from electric shock hazard.

Several new designs of photodiode assemblies have been developed and are being evaluated. A different type of interface box has been designed and built to allow integrating spheres at the ends of optical fibers to be used as the sensors. This is basically a different type of measurement, and work is under way to correlate the readings obtained by the two types of sensors. The spherical sensors are essential for monitoring light delivery for bladder cases, which are also monitored in real time.

During the past year, the software has been extensively modified to make it more user-friendly and to reduce the chance of operation error in setting up the system. Experimental work and mathematical modeling are being pursued to effect a more meaningful interpretation of readings taken with spherical sensors.

MAJOR FINDINGS: The HPD probe is useful for assessing the distribution of HPD and its selective concentration in malignant tissue. The light-monitoring system has been particularly useful for evaluating intralipid as a light-diffusing agent, and for quantitating the attenuation caused by the intralipid and blood. Useful information is also obtained on the light levels resulting from the operating room lights and surgeon's head lights. In addition to patient use, this system has been used in several laboratory studies to provide quantitative data.

SIGNIFICANCE: Data taken during dozens of human procedures have resulted in a substantial revision in the apportioning of light between different organs. In most instances, the real-time integrated values are now being used to control the duration of treatment. The results also clearly show the effect of leakage of blood into the intralipid, thus providing a sound basis for deciding when to change intralipid.

PROPOSED COURSE: Further software developments will make the system more versatile and easier to use. Additional theoretical and experimental work are needed to provide a more accurate relationship between measured values and the actual effective dose.

INVENTIONS: An invention report on a photodiode assembly has been filed.

PATENTS: A patent application for the handheld probe is in process.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10276-06 BEI															
PERIOD COVERED October 1, 1991 to September 30, 1992																	
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Microdialysis Probe Studies																	
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 40%;">Peter M. Bungay, Ph.D.</td> <td style="width: 30%;">Chemical Engineer</td> <td style="width: 30%;">CHES, BEIP, NCRR</td> </tr> <tr> <td>Robert L. Dedrick, Ph.D.</td> <td>Chemical Engineer</td> <td>CHES, BEIP, NCRR</td> </tr> <tr> <td>Paul F. Morrison, Ph.D.</td> <td>Physical Scientist</td> <td>CHES, BEIP, NCRR</td> </tr> <tr> <td>Kevin H. Dykstra, Ph.D.</td> <td>Chemical Engineer</td> <td>CHES, BEIP, NCRR</td> </tr> <tr> <td>Marianne Ekblom, Ph.D.</td> <td>Pharmacologist</td> <td>CHES, BEIP, NCRR</td> </tr> </table>			Peter M. Bungay, Ph.D.	Chemical Engineer	CHES, BEIP, NCRR	Robert L. Dedrick, Ph.D.	Chemical Engineer	CHES, BEIP, NCRR	Paul F. Morrison, Ph.D.	Physical Scientist	CHES, BEIP, NCRR	Kevin H. Dykstra, Ph.D.	Chemical Engineer	CHES, BEIP, NCRR	Marianne Ekblom, Ph.D.	Pharmacologist	CHES, BEIP, NCRR
Peter M. Bungay, Ph.D.	Chemical Engineer	CHES, BEIP, NCRR															
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Kevin H. Dykstra, Ph.D.	Chemical Engineer	CHES, BEIP, NCRR															
Marianne Ekblom, Ph.D.	Pharmacologist	CHES, BEIP, NCRR															
COOPERATING UNITS (if any) ETB, NIMH (W. Z. Potter, I. N. Mefford, J. K. Hsiao); Chemistry Department, Emory University (J. B. Justice, Jr.)																	
LAB/BRANCH Biomedical Engineering and Instrumentation Program																	
SECTION Chemical Engineering Section																	
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, MD 20892																	
TOTAL STAFF YEARS: 2.0	PROFESSIONAL: 1.7	OTHER: 0.3															
CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">_ (a) Human subjects</td> <td style="width: 33%;">_ (b) Human tissues</td> <td style="width: 33%; text-align: center;">X (c) Neither</td> </tr> <tr> <td colspan="3">_ (a1) Minors</td> </tr> <tr> <td colspan="3">_ (a2) Interviews</td> </tr> </table>			_ (a) Human subjects	_ (b) Human tissues	X (c) Neither	_ (a1) Minors			_ (a2) Interviews								
_ (a) Human subjects	_ (b) Human tissues	X (c) Neither															
_ (a1) Minors																	
_ (a2) Interviews																	
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Microdialysis probe technology provides access to tissue interstitium for either sampling of diffusible tissue constituents or delivery of bioactive substances. However, the relationship of the probe perfusate concentrations to their tissue counterparts is a complex function of solute molecular weight, solute physicochemical properties, tissue properties, probe membrane properties, probe geometry and perfusion rate, and the trauma of probe insertion into the tissue. These dependencies are being studied in order to improve the quantitative usefulness of the technology. The focus of applications is the brain in connection with diseases such as Parkinson's and AIDS. Endogenous solutes of interest include cyclic adenosine monophosphate (cyclic AMP) and dopamine. Exogenous substances employed as marker solutes or pharmacological agents are acetaminophen, AZT, and sucrose. Mathematical modeling is being used to describe solute transport within the probe and surrounding medium. Validation experiments involve quantitative autoradiography, histology, and chemical assay of tissue surrounding the probe, as well as measurement of probe perfusate concentrations.																	

RELEVANT BEIP PROJECTS: 88-111, Microdialysis Probe Studies; 91-140, Intrathecal AZT for AIDS Dementia; 91-157, Metabolic Network Analysis of G-Protein Signal Transduction.

OBJECTIVES: The first set of general objectives is to understand and better describe the factors that affect microdialysis performance in order to improve the usefulness of the technology and expand its capabilities. The term *performance* is used here to refer to the relationship between microdialysis perfusate concentrations and those in the medium surrounding the probe, i.e., the tissue concentrations in *in vivo* situations. A second set of objectives concerns specific applications in neuroscience research and studies of diseases such as Parkinson's and AIDS dementia.

METHODS EMPLOYED: A microdialysis probe contains a synthetic membrane, typically in a cylindrical capillary configuration. The inside of the probe is perfused with a dialysate medium. The effluent perfusate is either passed by a detector or collected for subsequent assay of the constituents that have crossed the membrane to (or from) the medium bathing the external surface of the membrane.

Unless the perfusion medium (dialysate) is allowed to equilibrate with the external medium, the concentrations of solutes in the perfusate will differ from the corresponding exterior concentrations. This phenomenon occurs because of gradients in solute concentration within the dialysate, membrane, and tissue inherent to the diffusional exchange of solute between the tissue and the dialysate. In general, empirical *in vitro* calibration of a probe cannot be relied upon in *in vivo* applications because tissue characteristics with respect to transport, binding, metabolism, vascularization, and trauma of probe insertion are difficult to mimic *in vitro*.

Mathematical modeling is being used for simulating solute transport within the blood, tissue, and probe compartments. The probes are characterized in *in vitro* studies. *In vivo* studies in the brains of anesthetized rats utilize model compounds to aid in understanding transport through tissue and the perturbation caused by the presence of the probe.

Available assay technologies include HPLC, electrochemical and spectrophotometric detection, and scintillation counting.

In addition, a quantitative autoradiography technique has been developed to determine the spatial variation in solute concentration in the tissue surrounding the probe. In this technique, radiolabeled solute is included in the dialysate flowing into the probe. At the end of a desired infusion interval, the animal is decapitated and the head is rapidly frozen. Thin tissues cut perpendicular to the probe are exposed to photographic film, along with calibrated radiation standards. The concentration profile for the radiolabel is determined from

the variation in light absorbance of the developed film. The shape of the profile and its change with duration of infusion provide information to complement measurements of the change in solute levels between the inflowing and outflowing dialysate.

MAJOR FINDINGS: General mathematical treatments for quantitative microdialysis have been developed. These treatments involve descriptions for solute transport in terms of dialysate, membrane, and external medium mass transfer resistances. For *in vivo* applications, the description for solute transport through tissue includes the following types of processes that affect solute levels: diffusion, generation, catabolism, extracellular-to-intracellular exchange, and extracellular-to-microvascular exchange. For situations in which these processes could be modeled by zero-order or linear first-order kinetics, it was possible to obtain explicit solutions to the governing equations for transient as well as for steady-state conditions. For *in vitro* applications, expressions derived for quiescent and well-stirred external media demonstrate how strongly dialysate concentration can be affected by convection in the external medium.

The explicit steady-state solution was evaluated for the sampling of tritiated water from rat brains, and the results were used to compare theoretical predictions to quasi-steady-state data from the literature. Excellent agreement was obtained between theory and experimentation for the variation in dialysate effluent concentrations with dialysate flow rate. The theory clarified the origin of the discrepancy between *in vivo* and *in vitro* microdialysis measurements.

An annular flow apparatus for *in vitro* probe characterization was constructed, in which the degree of convection in the external medium can be varied in a controlled and describable manner. Studies have been performed with this apparatus, using acetaminophen as the marker solute.

Transient *in vivo* experiments were performed in which acetaminophen was administered to rats by bolus intravenous injections. In one set of experiments, microdialysis probes were used to sample acetaminophen from the caudate; in another, anesthetized rats were sacrificed at various postinjection time points to obtain brain tissue samples for assay. Very good agreement was obtained between the predictions from the linear transient theory and observed time courses of both the dialysate effluent and total tissue drug concentrations.

Thus, specific models derived within the proposed general modeling framework have been tested for both transient and steady-state probe operation. Additional simulations were performed for a variety of small solutes of interest: cisplatin, dihydroxyphenylacetic acid (DOPAC), glucose, and adenosine.

The mathematical modeling efforts have been extended in several ways. One way was to develop simplified solutions in cylindrical geometry for the dialysate and membrane compartments, collapsed into a boundary condition for the external medium compartment. Whereas the original cylindrical coordinates transient solution was limited to low values of dialysate extraction fraction, the simplified solutions are valid over a wide range of extraction fractions. In addition, a corresponding transient solution in spherical coordinates was developed to assist in examining the importance of end effects. All of these model variations are restricted to one solute, and the extracellular and intracellular spaces have been coupled by the assumption of rapid exchange between these compartments. The various models were incorporated into MICRODIAL, a user-friendly Macintosh simulation program.

A series of *in vivo* validation experiments was conducted involving the administration of the extracellular marker [^{14}C]-sucrose through microdialysis probes into rat caudate. Extraction of solute from the dialysate was indicated by the inflow-outflow solute level difference. Autoradiography was used to follow the radial variation in tissue radioactivity. Comparisons between the measurements and the predictions from the mathematical models for both extraction and tissue profiles were consistent in suggesting that an edematous region of 35% to 40% extracellular volume fraction surrounded the probes. The edema appeared to extend beyond the approximate 3-mm-diameter interval over which the sucrose levels were detectable.

These quantitative microdialysis techniques were then used to study transport within the brain of the AIDS drug, zidovudine (AZT). These studies were undertaken in support of a proposed clinical trial for intraventricular administration of AZT to patients suffering from AIDS-related dementia. The objective was to assess whether therapeutic levels of the drug could be attained throughout the brain by this route of administration. These studies examined AZT metabolism in rat brain and the influence of systemically administered probenecid. The results implied that AZT is actively transported out of the brain parenchyma to the microvasculature, and that this mechanism is responsible for the limited penetration of systemically administered AZT into the central nervous system (CNS). Concomitantly, CNS penetration can be enhanced by inhibiting the active transport.

In the course of preparing to perform microdialysis measurements on dopamine and its common metabolites, a potential new metabolite of dopamine was identified; its production from dihydroxyphenyl-acetic acid (DOPAC) is catalyzed by copper and manganese ions. This new compound is of special interest, because poisoning by these two metals produces lesions similar to those observed in Parkinson's disease.

A transient model was developed for microdialysis of dopamine and the metabolites DOPAC, 3-methoxytyramine (3-MT) and homovanillic acid (HVA). The model contains intracellular compartments for

free cytosolic, inactive bound, and vesicular releasable dopamine, in addition to extracellular dopamine. Nonlinear saturable expressions were employed for the extracellular-cytosolic and cytosolic-vesicular dopamine exchange processes, and for dopamine conversion to 3-MT. Illustrative simulations for basal conditions suggest dopamine is sampled from within 20 μm of the probe surface, while DOPAC and HVA are sampled from substantially further (500 μm) because DA is much more rapidly cleared from the interstitium than the metabolites. In simulations for the effect of inhibition of uptake and metabolism on solute concentrations and probe performance, the model qualitatively reproduces the major features of the following experimentally-observed responses: dopamine uptake inhibition by cocaine, inhibition of catecholamine O-methyltransferase (COMT) by tropolone, and inhibition of monoamine oxidase (MAO) by pargyline.

Microdialysis studies of cyclic adenosine monophosphate (cyclic AMP) have been conducted. The evidence from the autoradiographic profiles indicates strong uptake and trapping intracellularly by nonlinear saturable processes. Metabolism is involved, since HPLC separation of labeled constituents from digested tissue surrounding the probes yielded AMP, ADP, and ATP but no detectable cyclic AMP. Detectable levels of labeled adenosine were found in the outflow dialysate samples, in addition to cyclic AMP, but no AMP. The studies to date are inconclusive with respect to which specie or species are transported from the extracellular to the intracellular compartments.

SIGNIFICANCE: Considerable interest is being shown in microdialysis because of its potential application to a variety of tissues and a wide range of solutes. The technology is not solute-specific, and perfusate samples are free of macromolecules; consequently, various separation and analysis techniques can be employed in the subsequent sample processing. However, the empirical manner in which the technology has been applied has limited its utility and hampered interpretation of measurements. The mathematical models developed in this project provide a rational basis for planning and interpreting microdialysis experiments in ways that would not be feasible otherwise.

PROPOSED COURSE: Work will continue on the application of the dopamine model, since the results to date suggest ways in which the mathematical model and quantitative experimental techniques can be utilized together to improve the interpretation of microdialysis measurements in dopaminergic tissue. The observation of substantial edema in the vicinity of acutely implanted probes should be followed up with studies investigating changes that occur over longer time intervals after probe implantation. Many investigators wait for a day after implanting probes before beginning to make measurements. The validity of the assumption that the tissue returns to normal within a day after suffering the trauma of probe insertion needs further testing. Desirable extensions of the modeling studies include incorporating

axial as well as radial diffusion to investigate the importance of end effects.

PUBLICATIONS: Dykstra KH, Arya A, Arriola DM, Bungay PM, Morrison PF, Dedrick RL. Microdialysis study of zidovudine (AZT) transport in rat brain. Journal of Pharmacology and Experimental Therapeutics (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10285-06 BEI
PERIOD COVERED October 1, 1992 to September 30, 1993		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Calorimetric Investigation of DNA/Anthracycline Drug Interactions		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Courtney P. Mudd, Ph.D. Biomedical Engineer ACES, BEIP, NCRR		
COOPERATING UNITS (if any) IR NHLBI (D. Remeta); Department of Chemistry, Rutgers University (K. Breslauer)		
LAB/BRANCH Biomedical Engineering and Instrumentation Program		
SECTION Applied Clinical Engineering Section		
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, Maryland 20892		
TOTAL STAFF YEARS: 0.2	PROFESSIONAL: 0.2	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) _(a) Human _(b) Human X (c) Neither subjects tissues _(a1) Minors (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) After performing the first direct measurements of the binding enthalpies of the anthracycline drug daunomycin to DNA in the 10 to 20 micromolar range (where the monomeric form predominates), we extended the experiment to study the interaction with other DNA hosts at varying salt concentrations and temperatures. With a differential stopped-flow microcalorimeter, we were able to measure 120 of these enthalpies per day, with an uncertainty of only 3 microjoules. The enthalpy heats ranged from -20 microjoules (endothermic) to +50 microjoules (exothermic). The experiments have now been expanded to measure and compare a complete set of binding profiles for a series of DNA host complexes.		

OBJECTIVE: To measure the binding heats to daunomycin to a variety of DNA hosts with drug concentrations in the 10 to 20 micromolar range (the range of therapeutic dosage). The DNA hosts we used were poly d(A)*d(T), poly d(G)*d(C), salmon testes DNA, and calf thymus DNA. In addition, we varied the salt concentration from 20 mM to 500 mM in order to investigate the salt dependence of the interaction. We also varied the temperature from 0°C to 50°C in order to investigate the temperature sensitivity of these reactions. The binding enthalpies are good indicators of the drug's effectiveness in chemotherapy.

SIGNIFICANCE: With the sensitivity of our tantalum stopped-flow calorimeter, we can use sufficiently low drug concentrations to ensure that the monomeric form of the drug predominates. These experiments represented the first direct measurement of these heats at therapeutic levels. Previous measurements were performed at higher concentrations (for higher heat output), and corrections were used to account for drug aggregation at higher concentrations. Measurement of daunomycin's effectiveness at therapeutic levels is extremely important because of the drug's toxic side effects. At present, daunomycin is the drug used second most often in chemotherapy.

PROPOSED COURSE: Results indicate that the degree of salt dependence varies according to the type of DNA host used. Also, at these monomeric levels, the results show that the corrections used in earlier works (at higher concentrations) contained considerable errors in some cases. We have completed a study of the binding profiles to a variety of DNA host duplexes and their temperature sensitivities. The final phase of this project will be to investigate the salt-dependence effect.

PUBLICATIONS: Remeta DP, Mudd CP, Breslauer KJ. Thermodynamic characterization of daunomycin-DNA interactions: microcalorimetric measurements of daunomycin-DNA binding enthalpies. *Biochemistry* 1991;30:9799-9809.

Remeta DP, Mudd CP, Berger RL, Breslauer KJ. Thermodynamic characterization of daunomycin-DNA interactions: comparison of complete binding profiles for a series of DNA host complexes. *Biochemistry* 1993;32:5064-73.

DEPARTMENT OF HEALTHS AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10286-06 BEI
PERIOD COVERED October 1, 1992 to September 30, 1993		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) High-Speed Differential Stopped-Flow Calorimeter		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Courtney P. Mudd, Ph.D. Biomedical Engineer ACES, BEIP, NCRR Thomas R. Clem, Sr., BSEE Electronics Engineer EEES, BEIP, NCRR Stephen B. Leighton, Sc.D. Mechanical Engineer MES, BEIP, NCRR		
COOPERATING UNITS (if any) IR BC, NHLBI (R. Berger)		
LAB/BRANCH Biomedical Engineering and Instrumentation Program		
SECTION Applied Clinical Engineering Section		
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, Maryland 20892		
TOTAL STAFF YEARS: 0.2	PROFESSIONAL: 0.2	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) _ (a) Human subjects _ (a1) Minors _ (a2) Interviews _ (b) Human tissues X (c) Neither		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) A dual-channel, differential, high-speed, stopped-flow calorimeter has been constructed to study the effects of mixing speeds, flow velocities, and pressure drops on the thermal and optical time course of chemical reactions. The system uses a variable-speed flywheel and an electrically actuated clutch to transfer sufficient energy to the four drive syringes to mix reagent volumes of 200 microliters in 5 milliseconds or less. The inlet tubes from the drive syringes, as well as the mixer and the detection chamber, are kept at constant temperature between 25° and 50°C. An ultrafast thermistor (1 ms) is located in the detection chamber to measure the temperature rise during the reaction. The drive cam profile produces a constant acceleration of the syringes. An optically transparent mixing chamber allows optical measurement of the reaction. The observation tube is now made of cast urethane coated with a 1000-Å-thick layer of tantalum to prevent water vapor migration. The observation tube "floats" in O-ring seals to reduce shock loads during mixing. A linear optical encoder is used to measure the displacement of the drive syringes.		

OBJECTIVE: The original objectives of the single-channel instrument remain the same: (1) to mix two 200-microliter reagent samples in less than 5 milliseconds; (2) to measure the heat of reaction after mixing; (3) to measure the pressure drop across the mixer in order to evaluate mixing efficiency as a function of flow velocity; (4) to vary the mixing time from fewer than 10 ms to more than 100 ms; (5) to allow optical measurement of the reaction; and (6) to control the temperature of the reaction over the range of 25° to 50°C. After evaluation of the thermal sensitivity of the single channel, we found the predominant source of uncertainty to be temperature variations in the incoming reagents. The addition of a second channel connected in opposition (differential operation) to the first channel causes this incoming temperature variation to become a common-mode signal. The differential scheme significantly reduced the uncertainty and produced an enhanced differential sensitivity. However, the usable sensitivity was still limited by outside temperature gradients down the inlet/outlet tubing.

SIGNIFICANCE: The high-speed mixing capability of the instrument allows the direct measurement of reaction kinetics in the millisecond range, with both thermal and optical detection schemes. With the present sensitivity, we are not able to measure fast reactions down in the 10- to 50-microjoule range. The thermistor sensor is sufficiently sensitive to measure a heat in this range, but its performance is degraded by temperature fluctuations between the two cells that appear as a differential signal.

PROPOSED COURSE: To reduce the temperature fluctuations between the two cells, we have redesigned the cell holder to reorient the two cells. The new orientation increases the common-mode rejection by insuring that the temperature fluctuations appear equally in both cells. An improvement of a factor of 5 to 10 will result in a sensitivity sufficient to make the desired measurement.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10296-06 BEI									
PERIOD COVERED October 1, 1992 to September 30, 1993											
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Experiments with a High-Resolution Field-Emission STEM											
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">Richard Leapman, Ph.D.</td> <td style="width: 33%;">Physical Scientist</td> <td style="width: 33%;">OD, BEIP, NCRR</td> </tr> <tr> <td>Stanley Sun, Ph.D.</td> <td>Visiting Fellow</td> <td>OD, BEIP, NCRR</td> </tr> </table>			Richard Leapman, Ph.D.	Physical Scientist	OD, BEIP, NCRR	Stanley Sun, Ph.D.	Visiting Fellow	OD, BEIP, NCRR			
Richard Leapman, Ph.D.	Physical Scientist	OD, BEIP, NCRR									
Stanley Sun, Ph.D.	Visiting Fellow	OD, BEIP, NCRR									
COOPERATING UNITS (if any) LN, NINDS (S. B. Andrews); LSBR, NIAMS (F. Booy); Lehigh University (J. Hunt); Baylor College of Medicine (W. Chiu)											
LAB/BRANCH Biomedical Engineering and Instrumentation Program											
SECTION Electron Beam Imaging and Microspectroscopy Group											
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, MD 20892											
TOTAL STAFF YEARS: 1.5	PROFESSIONAL: 1.5	OTHER: 0.0									
CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">_ (a) Human subjects</td> <td style="width: 33%;">_ (b) Human tissues</td> <td style="width: 33%;">X (c) Neither</td> </tr> <tr> <td colspan="3">_ (a1) Minors</td> </tr> <tr> <td colspan="3">_ (a2) Interviews</td> </tr> </table>			_ (a) Human subjects	_ (b) Human tissues	X (c) Neither	_ (a1) Minors			_ (a2) Interviews		
_ (a) Human subjects	_ (b) Human tissues	X (c) Neither									
_ (a1) Minors											
_ (a2) Interviews											
SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.) Techniques for acquiring electron energy loss (EELS) maps in the BEIP field-emission STEM have been developed further. Computer-controlled compensation for specimen drift has been implemented to allow the long acquisition times (in excess of two hours) required to detect physiological concentrations of elements in cryosectioned tissue. This compensation has been achieved by periodically collecting fast elastic images during EELS mapping and using automated Fourier cross-correlation methods to determine shift vectors, which are then applied to correct for the drift. A new spectral acquisition mode has also been developed to reduce radiation damage in beam-sensitive biological specimens. After acquiring a fast digital elastic image, a binary mask of the area to be analyzed is generated and fed back into the digital scan generator. In this way, the EELS spectrum can be collected at low dose by distributing the incident electrons over any extended specimen area that has a complicated shape. In another development, EELS in combination with digital dark-field imaging has been used to determine the thicknesses of protein crystals, in order to perform more accurate three-dimensional electron crystallography so that data from different crystals can be successfully merged.											

OBJECTIVES: To develop parallel-EELS mapping techniques for obtaining elemental distributions in small subcellular compartments of rapidly-frozen, cryosectioned tissue; to investigate other high-resolution applications of EELS in the field-emission STEM.

METHODS EMPLOYED: The EELS data were obtained with a specially adapted magnetic sector spectrometer equipped with a photodiode array parallel detector. Frozen-hydrated cyosections of liver and brain were cryotransferred into the VG Microscopes STEM and freeze-dried. Spectrum-images were recorded with up to 128 x 128 pixels, each containing 1024 energy channels, by means of a PC486-based digital acquisition system. Processing was performed with both the PC486 system and a Macintosh II computer.

MAJOR FINDINGS: By applying cross-correlation techniques to 64 x 64 pixel, dark-field elastic images acquired rapidly (in approximately 0.2 seconds) at the end of each scan line in an EELS spectrum-image, it was possible to correct for specimen drift to better than 10 nm in two hours or more. By using another technique--multiple-area spectrum integration--it was possible to acquire low-dose EELS spectra from structures with complicated geometries. Development of a low-dose method for measuring protein crystal thicknesses to within one-quarter unit cell may provide an important step for three-dimensional structure determination by electron crystallography.

SIGNIFICANCE: Because of the automated specimen drift compensation, the parallel-EELS spectrum-imaging technique has such improved sensitivity that it should now be possible to detect even lower concentrations of second-messenger calcium in small subcellular organelles. It is anticipated that there will be important applications in the field of neurobiology, where subtle movements of calcium play an important role in synaptic activity.

Application of the low-dose thickness measurement to protein crystals should improve the feasibility of high-resolution structure determination by means of electron crystallography.

PROPOSED COURSE: To incorporate a parallel detector with faster readout and higher detective quantum efficiency in order to optimize collection of EELS images at lower energy losses and at lower doses.

PUBLICATIONS: Leapman RD, Hunt JA, Buchanan RA, Andrews SB. Measurement of low calcium concentrations in cryosectioned cells by parallel-EELS mapping. Ultramicroscopy 1993;49:225-34.

Ciliax BJ, Kirk KL, Leapman RD. Radiation damage of fluorinated organic compounds measured by parallel electron energy loss spectroscopy. Ultramicroscopy 1993;48:13-25.

Leapman RD, Brink J, Chiu W. Thickness measurement of glucose-embedded crotoxin complex crystals by electron energy loss spectroscopy. Ultramicroscopy 1993 (in press).

Leapman RD, Newbury DE. Trace elemental analysis at nanometer resolution by parallel electron energy loss spectroscopy. Anal Chem 1993 (in press).

Urquhart SG, Hitchcock AP, Leapman RD, Priester RD, Rightor EG. Analysis of polyurethanes using core excitation spectroscopy. Part I: model polyurethane foam polymers. J Polymer Sci B: Polymer Phys (in press).

Rightor EG, Mitchell G., Fischer D, Hitchcock AP, Urquhart SG, Leapman RD, Yeh A, Gland JL. Near edge spectroscopy of poly(ethylene terephthalate) and dimethylterephthalate: comparison of x-ray absorption and electron energy loss spectroscopy. J Phys Chem (in press).

Egerton RF, Leapman RD. Quantitative energy-loss spectroscopy (EELS). In: Energy-Filtered Transmission Electron Microscopy. Springer Series in Physics, 1993 (in press).

Leapman RD, Sun S, Andrews SB. Use of EELS to assess beam damage in biological microanalysis. Proceedings of the 27th annual meeting of the Microbeam Analysis Society. San Francisco: San Francisco Press, 1993 (in press).

Leapman RD, Hunt JA, Andrews SB. Biological microanalysis by parallel-EELS. Proceedings of the 27th annual meeting of the Microbeam Analysis Society. San Francisco: San Francisco Press, 1993 (in press).

Hunt JA, Leapman RD, Williams DB. Low-dose EELS and imaging strategies in the STEM. Proceedings of the 27th annual meeting of the Microbeam Analysis Society. San Francisco: San Francisco Press, 1993 (in press).

Newbury DE, Leapman RD. Trace nanoanalysis of nanophase magnetic ceramics by analytical electron microscopy with parallel detection electron energy loss spectrometry. Proceedings of the 27th annual meeting of the Microbeam Analysis Society. San Francisco: San Francisco Press, 1993 (in press).

Rightor E, Mitchell G, Leapman R, Urquhart S, Hitchcock A, Fischer D, Hunt J, Gland J. Polymer characterization in the STEM by EELS and comparison to synchrotron and gas phase EELS results. Proceedings of the 27th annual meeting of the Microbeam Analysis Society. San Francisco: San Francisco Press, 1993 (in press).

Newbury DE, Leapman RD. Detection limits for analytical electron microscopy with electron energy loss spectrometry and energy dispersive x-ray spectrometry. Proceedings of the 51st annual

meeting of the Electron Microscopy Society of America. San Francisco: San Francisco Press, 1993 (in press).

Leapman RD, Brink J, Chiu W. Thickness measurement of glucose-embedded crotoxin complex crystals by EELS. Proceedings of the 51st annual meeting of the Electron Microscopy Society of America. San Francisco: San Francisco Press, 1993 (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10303-05 BEI									
PERIOD COVERED October 1, 1992 to September 30, 1993											
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Assessment of Scratching in Biliary Cirrhosis Patients											
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">Thomas Talbot, M.S.</td> <td style="width: 33%;">Mechanical Engineer</td> <td style="width: 33%;">ACES, BEIP, NCRR</td> </tr> <tr> <td>Joseph Schmitt, Ph.D.</td> <td>Staff Fellow</td> <td>ACES, BEIP, NCRR</td> </tr> <tr> <td>Elijah Walker, M.S.</td> <td>Chief</td> <td>ACES, BEIP, NCRR</td> </tr> </table>			Thomas Talbot, M.S.	Mechanical Engineer	ACES, BEIP, NCRR	Joseph Schmitt, Ph.D.	Staff Fellow	ACES, BEIP, NCRR	Elijah Walker, M.S.	Chief	ACES, BEIP, NCRR
Thomas Talbot, M.S.	Mechanical Engineer	ACES, BEIP, NCRR									
Joseph Schmitt, Ph.D.	Staff Fellow	ACES, BEIP, NCRR									
Elijah Walker, M.S.	Chief	ACES, BEIP, NCRR									
COOPERATING UNITS (if any) LDS, NIDDK (N. Bergasa, E. A. Jones)											
LAB/BRANCH Biomedical Engineering and Instrumentation Program											
SECTION Applied Clinical Engineering Section											
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, Maryland 20892											
TOTAL STAFF YEARS: 1.5	PROFESSIONAL: 1.5	OTHER: 0.0									
CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;"> ☒ (a) Human subjects </td> <td style="width: 33%;"> ☐ (b) Human tissues </td> <td style="width: 33%;"> ☐ (c) Neither </td> </tr> <tr> <td> ☐ (a1) Minors </td> <td colspan="2"></td> </tr> <tr> <td> ☐ (a2) Interviews </td> <td colspan="2"></td> </tr> </table>			☒ (a) Human subjects	☐ (b) Human tissues	☐ (c) Neither	☐ (a1) Minors			☐ (a2) Interviews		
☒ (a) Human subjects	☐ (b) Human tissues	☐ (c) Neither									
☐ (a1) Minors											
☐ (a2) Interviews											
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Patients with primary biliary cirrhosis suffer from severe bouts of itching, manifested by chronic scratching. In order to evaluate the efficacy of experimental drugs that may relieve the itching, it is necessary to quantify the scratching. A piezoelectric foil cemented to the patient's primary scratching fingernail transduced mechanical vibrations generated by the scratching into an electrical signal, which was applied to the input of a miniature FM transmitter. After the signal was obtained with an FM receiver, it was processed by a custom-designed frequency counter, which in turn was interrogated and reset periodically by a personal computer. The study consisted of measurement periods of six to eight hours, both before treatment and after treatment with specific drugs. Each patient served as his or her own control.											

MAJOR FINDINGS: A scoring scheme was devised and implemented that provides an average scratching activity index per hour. This index was used in a large double-blind clinical protocol to determine the viability of Naloxone, an opiate receptor antagonist, as an ameliorating agent in the treatment of pruritus. The study, which is now complete, ran for four consecutive 24-hour periods, with two control (placebo) periods and two Naloxone periods. Neither the patients nor the investigators were aware of the contents of the intravenous infusate during the study. The results clearly demonstrated a statistically significant reduction in scratching activity while the Naloxone was being infused, indicating that the drug is effective in ameliorating the pruritus associated with cholestasis. The results of the study have been published. In addition, a new clinical protocol is underway, consisting of a double-blind study designed to investigate Nalmafene, the oral counterpart of Naloxone. Parallel analysis of certain underlying circadian rhythms that may unveil imprinted scratching behavior is also under way. Preliminary indications imply that scratching may be an independent biorhythm, as well as a manifestation of a dermal antagonist.

A major double-blind clinical trial is nearly complete that will determine the efficacy of the oral medication Nalmafene to ameliorate pruritus. This study also incorporated an orthodic device that attached to the patient's finger in a splint-like fashion. This device provided a mechanism to normalize the stiffness and attachment modality of the piezo film scratch sensors. This normalization is particularly important because the protocol required the monitoring of the scratching at specific times during the various phases of the drug-control or placebo-control regimens.

PROPOSED COURSE: Simultaneous recordings of ECG and scratching are being obtained so that we can investigate the frequency of the biorhythms that may be associated with scratching.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10305-05 BEI
PERIOD COVERED October 1, 1992 to September 30, 1993		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) CC Image Management System		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Ronald L. Levin, Sc.D. Biomedical Engineer MES, BEIP, NCRR		
COOPERATING UNITS (if any) MIS, CC (T. Lewis); DSB, DCRT (K. Kempner)		
LAB/BRANCH Biomedical Engineering and Instrumentation Program		
SECTION Mechanical Engineering Section		
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, Maryland 20892		
TOTAL STAFF YEARS: 0.1	PROFESSIONAL: 0.1	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews ☐ (b) Human tissues ☒ (c) Neither		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) In order to facilitate the viewing and analysis of biomedical images, the Clinical Center is in the process of designing and developing an Image Management System (IMS) to complement the current text-based Medical Information System (MIS). This new system will consist of the following four key components: (1) several different types of medical image gateways (MIGs); (2) an image archival and retrieval system (IARS) that has an MIS interface; (3) several different types of workstations (WS) possessing a common biomedical shell; and (4) an interconnecting communications network. The MIGs will permit digital radiological images (i.e., MR, CT, PET, etc.), nondigital radiological images (i.e., plane-film X-ray), and nonradiological images (i.e., video-microscopic images, EKG tracings, patient monitor information, etc.) to be converted to a common format, compressed in a loss-less manner, and automatically sent to the IARS. The IARS will permit not only the long-term archiving on optical media of the images coming from the MIGs, but also the retrieval of multiple modality images to local workstations in response to queries via the existing MIS data base, the new IMS data base, or individual departmental data bases. In the workstation area, we will be developing two or three different types of biomedical workstations. The key to this series of workstations is that they would utilize the same biomedical shell (i.e., user interface). Application programs for diagnostic imaging, stereotaxic surgical planning, radiation therapy planning, tumor staging, etc., will also be developed.		

OBJECTIVES: To facilitate the exchange of medically related images by permitting images from multiple sources to be accessed via a single data base and in a single file format, and to facilitate the manipulation and comparison of medically related images together with text-based medical records through the use of a specially designed biomedical workstation.

PROPOSED COURSE: This project is waiting for finalization of system design parameters and budgetary approval. Phase I (testing of prototypes of the major system components) will be started in FY93 through the implementation of the Multimodality Radiological Image Processing System.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10309-05 BEI
PERIOD COVERED October 1, 1992 to September 30, 1993		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Signal Conditioning and Data Acquisition System for Sleep-Deprivation Studies		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Courtney P. Mudd, Ph.D. Biomedical Engineer ACES, BEIP, NCRR		
COOPERATING UNITS (if any) CPB, NIMH (C. Everson)		
LAB/BRANCH Biomedical Engineering and Instrumentation Program		
SECTION Applied Clinical Engineering Section		
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, Maryland 20892		
TOTAL STAFF YEARS: 0.2	PROFESSIONAL: 0.2	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) An AT-type microcomputer with a resident 16-channel, 12-bit A/D board is used to measure and store signals from freely moving rats in a sleep-deprivation study. The computer acquires brain temperature signals from small, glass-encapsulated thermistors. The calibration values for each thermistor are stored in a separate calibration file and loaded into the program when it is started. A thermistor bridge circuit accommodates a tolerance of $\pm 20\%$ in the nominal thermistor resistance, and covers a range of 25° to 45°C with 0.01°C resolution and 0.04°C accuracy. The system also acquires a "core" or peritoneal temperature, using small RF transmitters that change frequency with temperature. The transmitter system is capable of 0.02°C resolution with 0.07°C accuracy. Custom software controls the sample acquisition rate, filtering linearization, calibration, and storage of the temperature signals. In addition to the temperature signals, the computer also acquires 2 EEG signals and an EMG. The custom software uses a weighted combination of these signals to determine the animal's sleep state, which can then be correlated with the temperatures. The system also has a provision to acquire the heart rate, but that feature has not yet been implemented. The A/D card runs in a "background" mode for 30-second intervals, with the data transferred directly to a specified memory location. This mode of operation frees the CPU for processing the previous interval's data and allows uninterrupted, or continuous, data acquisition. To reduce calibration errors in the transmitter temperature system, a computer-controlled water bath calibrator was developed that performs an automatic 3-point calibration of the transmitters to within 0.04°C . The use of this calibrator improved the transmitters' accuracy from 0.22°C to 0.07°C .		

OBJECTIVES: The processing and acquisition system has now been in operation for over a year. During this evaluation period, we have determined that the thermistor measurement system has an accuracy of $\pm 0.04^{\circ}\text{C}$ with a resolution of 0.01°C . The transmitter system, which comes with no calibration or sensitivity information, has a resolution of 0.05°C but an accuracy of only $\pm 0.22^{\circ}\text{C}$. The source of this error was traced to the water bath calibration scheme used to measure the sensitivity of the transmitters.

SIGNIFICANCE: The operation of this continuous data acquisition system will allow uninterrupted, long-term investigations into the interdependence of diet and sleep. Preliminary studies have shown a definite relationship between brain temperature and sleep deprivation. An accurate system for measuring both the brain temperature and the sleep state (vigilance) is indispensable for investigating this effect. Our brain temperature accuracy of $\pm 0.04^{\circ}\text{C}$ represents approximately a threefold improvement over the present system.

PROPOSED COURSE: The data acquisition software has been developed to handle a wide variety of anticipated errors without operator intervention. Since the data acquisition is continuous (24 hr/day), the software is designed to allow the computer to recover from a power interruption, determine where it should be in the data acquisition cycle, and restart the program at the correct time in the data acquisition cycle. We have also incorporated a feature that allows the operator to select certain epochs for complete (unprocessed) data download to a floppy disk for Fourier analysis of the fast signals (EEGs and EMGs). The next phase in this project will be to replace an existing system, based on an obsolete 8-bit A/D microprocessor, with this 12-bit system.

PUBLICATIONS: Mudd CP, Everson CA. A 16-channel microcomputer-controlled thermistor temperature measurement system for use in physiological studies ($35^{\circ}\text{--}40^{\circ}\text{C}$). In: Temperature: its measurement and control in science and industry. Toronto: American Institute of Physics, 1992;6(pt 2):1003-11.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10310-05 BEI
PERIOD COVERED October 1, 1992 to September 30, 1993		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Microcalorimeter Measurements of DNA-Protein Interactions		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Courtney P. Mudd, Ph.D. Biomedical Engineer ACES, BEIP, NCRR		
COOPERATING UNITS (if any) LMB, NIDDK (P. Ross); FCRF, NCI (Y. Tekeda); IR CH, NHLBI (R. Berger)		
LAB/BRANCH Biomedical Engineering and Instrumentation Program		
SECTION Applied Clinical Engineering Section		
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, Maryland 20892		
TOTAL STAFF YEARS: 0.2	PROFESSIONAL: 0.2	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews ☐ (b) Human tissues ☒ (c) Neither		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Several new proteins developed at the NCI modify the human immune system response and are of intense interest in the areas of organ transplants, AIDS, and chemotherapy, but are now available only in small amounts. Investigators at the NCI and NIDDK consulted the BEIP about measuring the heats of these small samples. We recommended using our tantalum stopped-flow microcalorimeter, which can measure heats of 10 microjoules or less in 80-microliter samples. By mixing the proteins with carefully constructed molecules of DNA and measuring the enthalpy, we can determine the protein-DNA bond interactions. The first experiments showed that we could measure heats in the range of 5 microjoules with a standard error of less than 0.5 microjoules. We have now completed the preliminary study of one of these proteins with two different DNA hosts at 25° and 37°C. We were able to measure a binding heat difference when one base pair on the DNA host was changed. Results show that the enthalpy is very low, indicating that the binding is almost exclusively entropy-driven. Based on the success of these measurements and the usefulness of the information acquired, we have constructed a refined version of the calorimeter with a reduced priming volume (250 microliters vs 1000) and an improved pre-equilibration scheme to increase usable sensitivity. Preliminary results indicate a threefold improvement in resolution (from 3 microjoules to 1) for enthalpy measurements. The reduced priming volume allows us to nearly double the number of useful runs obtainable from a 1-cc sample. We also discovered that the pressure-induced component of the flow artifact is not significant, and that pressure-relieving the sensors is of marginal value. The automated loading feature has not yet been incorporated into the instrument.		

OBJECTIVES: (1) To reduce the priming volume from 1 ml to approximately 0.2 ml in order to further conserve reagents and obtain more data from each sample. (2) To improve the pre-equilibration of the sample as it enters the calorimeter, as a means of improving baseline fluctuations and effective resolution. (3) To provide a means of computer-controlled loading of a series of samples to allow unattended operation over long periods of time.

SIGNIFICANCE: In studying these interactions, very often we change only one base pair in each experiment. Even a relatively small DNA molecule can require 30 to 40 experiments to study the protein's reactions. Automatic loading would allow unattended operation during nonworking hours. The reduced priming volume will conserve expensive reagents (\$4,000 to \$5,000 per experiment) by reducing the number of "lost" runs at the buffer/reagent flow interface and increasing the number of "good" runs. The improvement in the pre-equilibration will be especially important with the low priming volume system, to avoid allowing nonequilibrium-induced artifacts (that degrade baseline reproducibility) into the measurements.

PROPOSED COURSE: The redesigned calorimeter is now being evaluated. Preliminary tests indicate that with the new pre-equilibration scheme, baseline reproducibility is improved over the earlier design, and the priming volume is about 250 microliters. The final phase will involve interfacing the automated loading system to the instrument.

PUBLICATIONS: Taketa Y, Ross PD, Mudd CP. Thermodynamics of CRO protein-DNA interactions. Proceedings of the National Academy of Sciences USA, 1992;89:8180-4.

PATENTS: Mudd CP, Berger RL. US Patent 5,217,690: All Tantalum Stopped-Flow Microcalorimeter, June 8, 1993.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 RR 10313-05 BEI

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

High-Speed Multichannel Spectrophotometer

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Walter S. Friauf, MEE	Chief	EEES, BEIP, NCRR
Paul D. Smith, Ph.D.	Physicist	EEES, BEIP, NCRR
John Cole, MSEE	Electrical Engineer	EEES, BEIP, NCRR

COOPERATING UNITS (if any)

LCB, NHLBI (R. Hendler); CSL, DCRT (H. Frederickson)

LAB/BRANCH

Biomedical Engineering and Instrumentation Program

SECTION

Electrical and Electronic Engineering Section

INSTITUTE AND LOCATION

NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

1.0

PROFESSIONAL:

1.0

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human
subjects

☐ (b) Human
tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A number of commercial multichannel spectrophotometers using charge-coupled devices have recently been developed for general use in biomedical research. We have explored the possibility of improving both the speed and the accuracy of such instruments by exploiting other new devices.

OBJECTIVES: To develop a 92-channel spectrophotometer capable of sampling all channels at a maximum rate of 100,000 samples per second with 12-bit resolution.

METHODS EMPLOYED: A commercial spectrophotometer will disperse the light to two 46-element photodiode arrays. Each channel will integrate the signal between sample times, digitize it, and store it in a first-in, first-out register prior to digital multiplexing for transmission to a computer. The computer will provide full control over the sampling program, as well as over such experimental parameters as flow and flash photolysis; it will also handle all of the processing and permanent storage, will display data, and will perform various other functions.

SIGNIFICANCE: Achievement of the proposed specifications will open up a variety of new applications. The increased temporal resolution will permit investigation of early events in biochemical reaction kinetics that have been impossible to measure before. The ability to follow these processes with high accuracy and at multiple wavelengths enables investigators to track individual components within a complex reaction simultaneously.

PROPOSED COURSE: Design and construction are complete, and the instrument is in regular use. We are exploring the possibility of cooling the diode arrays, adding a programmable offset to each channel, and increasing the gain to provide improved resolution. We are also looking into the possible application of image intensifiers to extend the short-wavelength response of the instrument. A new commercial stopped-flow apparatus is being integrated into the system, and computer control of experimental parameters has been extended and made more user-friendly.

An invention report has been filed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 RR 10314-05 BEI

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pulsed Photodynamic Therapy

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Walter S. Friauf, MEE

Chief

EEES, BEIP, NCRR

John W. Cole, MSEE

Electrical Engineer

EEES, BEIP, NCRR

COOPERATING UNITS (if any)

ROB, DCT, NCI (A. Russo)

LAB/BRANCH

Biomedical Engineering and Instrumentation Program

SECTION

Electrical and Electronic Engineering Section

INSTITUTE AND LOCATION

NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

0.2

PROFESSIONAL:

0.2

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human
subjects

☐ (b) Human
tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Experimental evidence indicates that the efficacy of photodynamic therapy (PDT) is temperature-dependent. This insight creates the possibility of using a temperature gradient in the tissue to compensate partially for the rapid attenuation of light, thus allowing effective treatment to a greater depth. By pulsing the surface temperature at an appropriate rate and illuminating only during the cool phase, the treatment will occur at a selected depth during the warm phase, due to the propagation delay experienced by the temperature wave.

OBJECTIVES: To develop a technique to provide effective PDT to a greater depth than is now possible.

METHODS EMPLOYED: Computer simulation of one-dimensional thermal diffusion has been carried out, using a SPICE program. The geometry is reasonable for the surface of the peritoneum and for slab-shaped organs (e.g., the liver). The surface temperature was approximated by a trapezoidal waveform, representing the temperature control that could be achieved by changing intralipid.

MAJOR FINDINGS: An empirical relationship was determined between the frequency of the temperature variation, the depth, the two thermal parameters of the tissue, and the amplitude and phase of the temperature waveform in tissue. This was correlated with analysis based on transmission line theory. In principle, the result allows determination of the frequency needed to optimize treatment at a specified depth; however, the attenuation was so severe that a useful elevation in temperature at interior points could not be achieved. The analysis was extended to include both linear and nonlinear effects of circulation. Even then, the increase in temperature at interior points relative to the surface, although greater, was not sufficient to be particularly useful. Considerable benefit might still accrue through secondary effects, such as modification of the oxygen delivery rate and modification of optical parameters.

SIGNIFICANCE: PDT is limited to treating superficial regions because of the rapid attenuation of light in tissue. This new approach has the potential to ease this limitation somewhat.

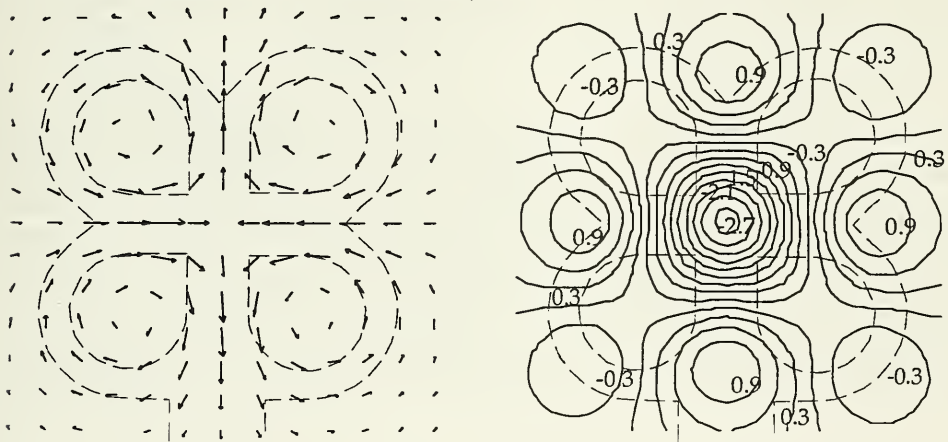
PROPOSED COURSE: The potential for exploiting secondary effects of temperature on circulation, and of circulation on oxygen availability, will be studied further. In addition, other methods of increasing the depth of treatment will be explored, including modification of the distribution profile of the photosensitive agent by iontophoresis, absorption, or other means; creation of a profile of an inhibitor, such as histidine or vitamin E; modification of the illumination schedule to exploit oxygen depletion; and use of pressure to inhibit oxygen replenishment near the surface. In addition, methods of improving light transmission through the skin will be explored, since hematoporphyrin derivative (HPD) apparently concentrates in skin, as well as in malignant tissue. Work on this project is expected to continue as new developments in inhibitors occur, and if there is a renewed attempt to apply PDT to breast cancer at a more sophisticated level.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10315-05 BEI
PERIOD COVERED October 1, 1992 to September 30, 1993		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) A Model of Magnetic Stimulation of a Nerve Fiber		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Bradley J. Roth, Ph.D. Senior Staff Fellow MES, BEIP, NCRR Peter Basser, Ph.D. Biomedical Engineer MES, BEIP, NCRR		
COOPERATING UNITS (if any) MNB, NINDS (L. Cohen, M. Hallett); Dept. of Neurology, SUNY, Brooklyn (P. Maccabee)		
LAB/BRANCH Biomedical Engineering and Instrumentation Program		
SECTION Mechanical Engineering Section		
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, MD 20892		
TOTAL STAFF YEARS: 0.2	PROFESSIONAL: 0.2	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) _(a) Human _(b) Human X (c) Neither subjects tissues _(a1) Minors (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) A model is presented to explain the physics of nerve stimulation by electromagnetic induction. Maxwell's equations predict the induced electric field distribution produced when a capacitor is discharged through a stimulating coil. A nonlinear cable model, containing active Hodgkin-Huxley elements, describes the response of the nerve fiber to this induced electric field. It is shown that the nerve fiber is stimulated by the gradient of the component of the induced electric field parallel to the fiber, which hyperpolarizes or depolarizes the membrane and may stimulate an action potential. Once the coil's position, orientation, and shape are given and the resistance, capacitance, and initial voltage of the stimulating coil are specified, this model predicts the resulting transmembrane potential of the fiber as a function of distance and time. Finally, it predicts complicated dynamics, such as action potential annihilation and dispersion. The model has been verified experimentally in humans. A new four-leaf coil design for magnetic stimulation of peripheral nerves has been developed and has been tested using <i>in vitro</i> experiments.		

OBJECTIVES: To increase our understanding of the physics of magnetic stimulation, and to design improved coils, by developing a mathematical model of the interaction of a nerve fiber with the induced electric field. Also, to develop new coil geometries for use during magnetic stimulation.

SIGNIFICANCE: Although the electric field induced in an arm can be calculated accurately, its interaction with a peripheral nerve had not been elucidated. Basic questions (such as the location of stimulation) must be resolved before magnetic stimulation can be fully utilized as a clinical tool. This model provides insight into the interaction of the electric field with the nerve, and provides a tool for predicting new coil designs.

MAJOR FINDINGS: The magnitude and time course of the gradient of the component of the induced electric field parallel to the nerve fiber determines if and where stimulation occurs. The location of the virtual cathode is sensitive to coil placement. A secondary virtual cathode and anode pair are predicted to exist for a circular coil. A four-leaf coil design is particularly appropriate for peripheral nerve stimulation, and its usefulness has been demonstrated experimentally.



The calculated electric field (left) and the derivative of the x-component of the electric field, dE_x/dx (right), produced by a four-leaf magnetic stimulation coil (dotted outline). The x-direction is horizontal, and dE_x/dx is given in units of mV/mm^2 .

PROPOSED COURSE: *In vitro* experiments with the four-leaf coil design are being completed, and a manuscript is being prepared for publication.

PUBLICATIONS: Nilsson J, Panizza M, Roth BJ, Basser P, Cohen L, Caruso G, Hallett M. Determining the site of stimulation during magnetic stimulation of a peripheral nerve. *Electroenceph Clin Neurophysiol* 1992;85:253-64.

Basser P, Wijesinghe R, Roth BJ. The activating function for magnetic stimulation from a three-dimensional volume conductor model. *IEEE Trans Biomed Eng* 1992;39:1207-10.

Basser P. Focal magnetic stimulation of an axon. *IEEE Trans Biomed Eng* (in press).

Basser P. A homogenized cable equation of a myelinated axon. *Med & Biol Eng & Comput* (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10316-05 BEI
PERIOD COVERED October 1, 1992 to September 30, 1993		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Calculation of Electric Fields During Magnetic Stimulation		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Bradley J. Roth, Ph.D. Senior Staff Fellow MES, BEIP, NCRR Walter S. Friauf, MEE Chief EEES, BEIP, NCRR		
COOPERATING UNITS (if any) MNB, NINDS (L. Cohen, M. Hallett); CE, NHLBI (R. Turner); Ballou Senior High School (S. Momen)		
LAB/BRANCH Biomedical Engineering and Instrumentation Program		
SECTION Mechanical Engineering Section		
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, MD 20892		
TOTAL STAFF YEARS: 0.1	PROFESSIONAL: 0.1	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews ☐ (b) Human tissues ☒ (c) Neither		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Magnetic stimulation is a new technique for activating neurons that is both painless and noninvasive. Stimulation occurs when a brief current pulse is passed through a coil placed near the neuron, producing an electric field by electromagnetic induction. The goal of this project is to develop mathematical models to calculate the electric field induced in the human body during magnetic stimulation. Two models are developed in detail: (1) a model of the stimulation of a peripheral nerve in the arm, and (2) a model of the stimulation of the brain. Techniques to solve the inverse problem of determining the appropriate coil geometry for producing a specified electric field distribution are also studied.		

OBJECTIVES: To calculate the electric field distribution in the body during magnetic stimulation.

SIGNIFICANCE: Although the use of magnetic stimulation is growing rapidly, the technique has been applied clinically without a complete theoretical understanding of the induced electric field distribution. If this distribution can be calculated numerically, some theoretical insight will be provided to guide future applications of the technique. In addition, these calculations will aid in the development of more focal coil geometries.

MAJOR FINDINGS: During magnetic stimulation, a charge distribution arises on the tissue-air surface that partially shields the interior of the body from the stimulus. Taking the presence of this charge distribution into account is essential to the correct calculation of the electric field. Generally, this shielding effect is more pronounced for coils that are oriented perpendicular to the tissue-air surface. The peak electric field occurs near the surface of the tissue. It is possible to determine the current distribution outside the head that is needed to induce a specified electric field distribution within the head. This determination should be useful for coil design.

PROPOSED COURSE: Techniques are being developed to perform the inverse problem: Given a desired electric field in the head, what is the coil geometry required to produce that field?

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10318-05 BEI
PERIOD COVERED October 1, 1992 to September 30, 1993		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Apple Macintosh II-Based Image Processing Workstation		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Michael Unser, Ph.D. Visiting Scientist OD, BEIP, NCRR		
COOPERATING UNITS (if any) None		
LAB/BRANCH Biomedical Engineering and Instrumentation Program		
SECTION Office of the Director		
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, MD 20892		
TOTAL STAFF YEARS: 0.5	PROFESSIONAL: 0.5	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) _ (a) Human _ (b) Human X (c) Neither subjects tissues _ (a1) Minors _ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Personal computers (such as the Macintosh II) offer very cost-effective hardware configurations for simple image processing tasks. However, programming them using conventional techniques is notoriously difficult, due to the high level of sophistication of the user interface. The main purpose of this project is to devise powerful development tools to facilitate the development of specialized image processing software for such workstations. The first step was to create a simple image processing shell that is easy to modify for creating specialized applications. The main feature is that all complex interactions with the user interface are handled automatically; the programmer need only be concerned with the application-specific procedures. The second step was to develop a comprehensive set of basic image processing modules. Our software library now includes about 275 subroutines that perform image processing tasks (e.g., filtering, multiresolution and wavelet decompositions, edge detection, point transformations, and binary operations).		

OBJECTIVES: To develop an extensive software library that can be used to facilitate and standardize the development of user-friendly image processing programs on the Macintosh II computer family.

METHODS EMPLOYED: Most software has been written in Absoft FORTRAN V2.4, and more recently in C using Think C V5.0. The integration of basic subroutines into application-specific programs is centered around the use of the FACEIT shell, which substantially accelerates and simplifies the development process. Our image processing software library currently includes a relatively large selection of point operators (histograms, nonlinear transformations, simple arithmetics, statistics, etc.), neighborhood operators (moving average and Gaussian smoothers, Laplacian, convolution, median, and morphological filters, adaptive least squares, and B-spline filters), wavelet transforms and image pyramids, and binary operations (automatic thresholding, thinning, expansion, erosion). There is also a collection of input/output subroutines for reading and writing images in TIFF format, display, and interactive specification of points, lines, or regions of interest.

MAJOR FINDINGS: This new programming environment has cut down development time substantially and improved reliability. Special-purpose image processing software that was developed based on this strategy includes Mecho (automated extraction of serial myocardial borders from M-mode echocardiograms), GelFit (analysis of two-dimensional gels in a size-mobility map), μ Edge (a comprehensive set of edge-detection algorithms), μ Tongue (detection of the tongue surface from ultrasound images), and μ Register (iterative multiresolution image registration). FLIPS (Flexible Language-based Image Processing System) and MULTIMAGE integrate most of the currently available subroutines.

SIGNIFICANCE: The availability of efficient development tools is essential to setting up small collaborative projects with other researchers in the NIH community. There are several advantages in designing an image processing system based on a Macintosh II computer. First, this type of computer is now widely available for a variety of personal computing tasks, which means that the purchase of new equipment is usually not necessary. Second, the Macintosh II stands as a very cost-effective alternative to conventional image processing systems. Third, all Macintosh programs are designed to be very user-friendly, which makes them easy to use and facilitates their dissemination. Finally, most data formats are standardized, which greatly facilitates the exchange of information among different applications. Custom programs and general-purpose applications (statistics, graphs, etc.) can be combined very efficiently to solve a specific problem.

PROPOSED COURSE: We will extend the present environment by adding new image processing functions. We are also planning to translate some of our older FORTRAN subroutines into C for software development in a UNIX environment.

PUBLICATIONS: Unser M. Efficient dyadic wavelet transformation of images using interpolation filters. In: Proceedings of the International Conference on Acoustics, Speech, and Signal Processing. Minneapolis: IEEE, 1993;149-52.

Unser M. Texture discrimination using wavelets. In: Proceedings of the IEEE Conference on Computer Vision & Pattern Recognition. New York: IEEE, 1993;640-1.

Unser M, Aldroubi A, Gerfen CR. A multiresolution image registration procedure using spline pyramids. In: Proceedings of the SPIE, Vol. 2034: Wavelet applications in signal and image processing (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10322-05 BEI
PERIOD COVERED October 1, 1992 to September 30, 1993		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Data Acquisition System for an Ultrahigh-Resolution Dedicated STEM		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Carol R. Swyt, M.S. Physical Scientist OD, BEIP, NCRR		
COOPERATING UNITS (if any) CAC, NIST (C. Fiori, R. Myklebust)		
LAB/BRANCH Biomedical Engineering and Instrumentation Program		
SECTION Electron Beam Imaging and Microspectroscopy Group		
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, Maryland 20892		
TOTAL STAFF YEARS: 3.0	PROFESSIONAL: 3.0	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews ☐ (b) Human tissues ☒ (c) Neither		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) An automated data acquisition system is being developed for the Vacuum Generators HB 501 ultrahigh-resolution dedicated scanning transmission electron microscope (STEM). The new analytical capabilities of this class of electron microscope require fresh approaches to computerization of data acquisition and analysis. Available commercial systems have been limited in both the kinds of data that can be acquired concurrently and the algorithms for processing the data. As part of this system, a desktop data acquisition and analysis program, called Desk Top Spectrum Analyzer and X-Ray Database (DTSA), has been developed for the Macintosh II (and higher) class of computers. The data acquisition is accomplished utilizing specially modified commercial plug-in bus cards and control software routines that interface to the control and analytical software of DTSA. A patent, to be held jointly by the NIH and NIST, is pending on this analytical tool. The DTSA is available from the Office of Standard Reference Data (OSRD), National Institute of Standards and Technology, for a modest fee. A copyright has been obtained by the OSRD.		

METHODS EMPLOYED: The software is written in MPW Pascal. The user interface utilizes the full range of Macintosh utilities, including windows, menus, dialogs, and color display. Algorithms for all of the relevant physics for the generation of x-rays in a thin or bulk specimen by an electron beam, and detection by a lithium-drifted silicon detector, have been incorporated into the program. The user may select from a number of the most widely-used cross sections for x-ray generation, and may input microscope, specimen, and detector characteristics that determine the final spectrum distribution. Any of the functions calculated, such as the continuum distribution and characteristic peaks before and after convolution with the detector function, may be displayed in one of the ten real-valued overlaid "spectrum displays." The information in the x-ray database is available to the user via a menu that provides relative intensities with the lines, edges, or satellites specified in the user-selected units of eV, mm, Å, or (for wavelength-dispersive x-ray spectrometry) $\sin(\theta)$ for the crystal spectrometer chosen. Both linear and nonlinear spectral peak unravelling methods are available, and quantitation schemes for thin and bulk biological, geological, and other specimens have been provided. X-ray spectra acquired by wavelength-dispersive x-ray spectrometry may be imported, converted to energy display, and processed using any algorithm in DTSA. Among the other routines provided are qualitative analysis, spectrum calibration, beam energy calculation based on the Duane-Hunt limit, peak stripping, background subtraction, Fourier transforms, spectral smoothing, and simple scaling and summing of distributions. Additionally, x-ray spectral data acquired with other systems may be transferred to the program and converted to internal format for processing. Any displayed distribution may be sent directly to a printer or stored in a file. Menus are provided for supplying full notebook annotation of data files. The results of spectral unravelling and quantitation may be reported in any one of the common spreadsheet formats for statistical analysis and plotting, and output to hard copy with full file annotations.

An interface to the acquisition control software is provided in the program. The parameters for acquisition (such as data collection time, characteristics of the detector system, etc.) are supplied via the DTSA program to the acquisition software. Information such as dead time, real time, and data input rate are constantly displayed. As data are acquired, they are transferred to the Macintosh memory and are available for constantly updated display as a spectrum on the Macintosh screen, and for processing with any algorithm in the DTSA program.

Version 2 of the program was released in July 1993 by the Office of Standard Reference Data, National Institute of Standards and Technology. A number of commercial x-ray analyzer manufacturers have negotiated to offer DTSA as an option with their systems or to utilize the x-ray database in their own analyzer systems.

SIGNIFICANCE: Though designed for the EBIMS VG HB 501 STEM, the DTSA provides analysts in any laboratory with an easily customized means of obtaining x-ray data, spectrum or image, from any electron microscope equipped with the appropriate detectors. The cost is about one-tenth that of traditional energy-dispersive x-ray systems. In addition, it provides the full capabilities for data analysis developed over the years in the EBIMS group, and for data presentation and statistical analysis developed for the Macintosh computer. It is the only analyzer system to provide the means to apply these capabilities to data acquired with wavelength-dispersive x-ray analysis systems. These capabilities are implemented in the typically user-friendly Macintosh environment. The program provides the analyst with a set of tools to simulate, analyze, and better understand the generation and detection of x-rays from his/her sample, and therefore to design more informative and efficient experiments. This work can be done on the desktop, freeing the electron-beam instrument for productive data acquisition.

PROPOSED COURSE: Modifications will be made in the algorithms to provide capabilities for x-ray fluorescence data similar to those now provided for electron beam x-ray data. Image acquisition and processing routines, as well as wavelength-dispersive x-ray spectrum and image acquisition and analysis capabilities, are being developed.

Negotiations are under way between commercial manufacturers of electron microscopes and x-ray analytical equipment, and the NIST and the NIH, for the rights to distribute and otherwise use the program.

PUBLICATIONS: Fiori CE, Swyt CR. Is spectrometer resolution or system count rate performance more important in quantitative energy-dispersive x-ray microanalysis? In: Bailey GW, Bentley J, Small JA, eds. Proceedings of the 50th annual meeting of EMSA. San Francisco: San Francisco Press, 1992;1770-1.

Fiori CE, Swyt CR. Is spectrometer resolution or system count rate performance more important in quantitative energy-dispersive x-ray microanalysis? A study using generated spectra. Microbeam Analysis 1992;1:89-95.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10324-05 BEI
PERIOD COVERED October 1, 1992 to September 30, 1993		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) In Vitro Hemodynamic Models for Cardiovascular Studies		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Robert J. Lutz, Ph.D. Chemical Engineer CHES, BEIP, NCRR Travis Jones, Ph.D. Chemical Engineer CHES, BEIP, NCRR		
COOPERATING UNITS (if any) IR CB, NHLBI (J. Peacock); ROB, DCT, NCI (A. Epstein); LDDR, OD (W. Pritchard); Georgetown University Hospital (S. Lossef, T. Takeda)		
LAB/BRANCH Biomedical Engineering and Instrumentation Program		
SECTION Chemical Engineering Section		
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, MD 20892		
TOTAL STAFF YEARS: 3.0	PROFESSIONAL: 2.0	OTHER: 1.0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews ☐ (b) Human tissues ☒ (c) Neither		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Physical models of vascular systems are being used extensively in our laboratory to study a variety of phenomena related to hemodynamics. During the past year, efforts have been directed to projects in the following categories: (1) a study of intra-arterial drug delivery, using a glass model of the pelvic arteries; (2) a study of the effect of fluid shear on the adhesion of monocytes onto cultured endothelial cells; (3) numerical analysis of flow characteristics in branching vessels; (4) investigation of mechanical strength of several types of cardiovascular stents; and (5) study of the injection characteristics and mixing properties of several angiographic catheters, using a glass model of the human aorta.		

OBJECTIVES: (1) Intra-arterial administration of drug solutions can provide a regional advantage to cancer therapy by yielding a high local concentration of drug in a tumor region while maintaining lower, less toxic systemic levels. Radiation-sensitizing drugs, in combination with radiation, have been used with good success in treating gynecological cancers. To achieve optimum success in treating cervical cancer, the radiosensitizing drug would be delivered only to the tumor-bearing region and would avoid other sensitive tissues around the uterus and rectum. *In vitro* flow models of the cervical artery network allow us to study proper placement of infusion catheters in the uterine artery and to assess different methods of drug delivery that will insure adequate distribution of the drug to the tumor site while minimizing levels elsewhere.

(2) Vascular endothelial cells are important mediators in numerous biological processes, including regulation of vascular tone, inflammation, and atherogenesis. Cultured endothelial cells have been shown to have unique responses to fluid shear stresses *in vitro*, as they do *in vivo* (e.g., morphological alterations and expression of biological components that affect cell adhesion and receptor binding). An early event in atherogenesis is adhesion of monocytes to the arterial wall. A cell culture apparatus that exposes endothelial cells to controlled fluid shear stresses can assess the relationship between hemodynamics, cell adhesion, and atherogenesis. Such an apparatus can also be used to study shear stress effects on the production of biological components from the endothelial cells.

(3) One objective of the experimental turbulence studies was to determine what conditions of total flow, frequency of flow oscillation, and stroke volume would cause a transition from laminar to turbulent flow in straight and branched tubes. We propose to use state-of-the-art numerical analysis computer programs to corroborate our experimental studies. This information is relevant to gaining a better understanding of the flow conditions that could lead to turbulence in the more complicated geometry of the coronary vasculature. The presence of turbulence in the coronary circulation is suspected as a cause of thrombus formation around coronary artery stents inserted for structural support following angioplasty. Another objective was to determine the effect of tube branching on the generation of flow instabilities and turbulence. Two simple 45° branching configurations were constructed, with parent-to-daughter area ratios of 1 to 2 and 1 to 1.15. The results of the branching studies were compared to data collected in a casted model of a pig coronary artery. Models of 75% and 90% stenoses in straight tubes were studied as simple simulations of coronary restenosis and used to determine the onset of turbulence in pulsatile flow conditions. The data from these models have been used to develop correlations that predict the onset of turbulence and to estimate friction factors. Velocity profile data have also been generated and compared to theory for a range of flow from laminar to turbulent.

(4) Angioplasty is a frequently used, minimally invasive procedure for opening occluded or stenosed arteries. The high incidence of restenosis following angioplasty (30%-40%) is problematic. To maintain patency and inhibit restenosis, cardiovascular stents have been employed. These stents are cylindrical-shaped scaffolding devices which can be deployed transluminally in arteries using special catheters. Several types of stents are now being manufactured. It is important to know the mechanical strength of these stents in order to determine their ability to withstand the restenosis forces of the arterial wall and to assess which stents are suitable for use in various arterial regions. It is also useful to study the effects of deployed stents on the blood flow characteristics, since it is believed that flow disturbances caused by the stent against the arterial wall can lead to clot formation and eventual arterial occlusion in the presence of the stent.

(5) Angiography is an important tool in the diagnosis of cardiovascular disease. A proper angiographic evaluation relies on homogeneous distribution of contrast agent delivered from an angiographic catheter. Many types of catheters are available for clinical use that have different shapes and use different injection rates. There are few systematic studies that evaluate the injection characteristics of angiographic catheters; even more critically, there are few convenient model systems for performing these studies. We have developed an *in vitro* model of the human aorta, along with a pulsatile flow system that can assess the homogeneity of delivery of angiographic contrast material to branch vessels of the aorta.

METHODS EMPLOYED: (1) A flow model of the ileofemoral arterial network of a female human, including branches of the uterine and cervical arteries, was fabricated from glass using published anatomical illustrations as a guide. The model is connected to a flow system that generates pulsatile flow waves. The numerous branches of the pelvic artery model are connected to a collection device that allows individual sampling of the effluent fluid (simulated blood) from these branches. A red dye is injected via a catheter at various locations in the arterial system, and fluid is sampled from the distal branches to determine the distribution of dye by measuring dye concentration spectrophotometrically. Various injection sites have been tested. A comparison was made between a slow, steady infusion and a rapid, intermittent bolus infusion that is generated by a specially modified Medrad infusion pump. This pump, referred to as the DPPI pump, synchronizes the injection with the diastolic phase of cardiac output. Flow visualization studies are recorded on videotape to maintain a visual record of the infusion experiments.

(2) A cell-culture chamber was fabricated as a parallel-plate design. Two stainless-steel plates are separated by a thin Teflon sheet to produce a gap for flow of culture media through the chamber. Two windows are cut out of the center portion of the rectangular plates, and two glass microscope slides are secured in

the window openings to allow observation of the cells, which are seeded on the luminal surface of the bottom window. The flow chamber is connected by silicone tubing to glass reservoirs, one of which is elevated to provide inlet head for steady flow of fluid. The other serves as the effluent collection tank for eventual recirculation of the fluid (medium) via a peristaltic pump to the head tank. The entire system, including an Olympus phase contrast microscope, is housed in an incubation chamber made of styrofoam board and maintained at a constant temperature of 37°C by a regulated hot-air blower. Fluid shear stresses are regulated by adjusting the head-tank level, adjusting flow resistance with pinch clamps, or changing the gap spacing of the parallel-plate chamber.

(3) The experimental studies of the transition of flow to turbulence were conducted in a series of straight and branched tubes, as well as in a cast of a human coronary artery. These models were made of plastic or silicone rubber. Hot film probes, electrochemical shear probes, and laser Doppler anemometry were techniques used to measure wall shear stress and velocity in the physical models. A state-of-the-art numerical algorithm called FIDAP was installed on our IBM-PC. The program uses a finite element method that allows the user to develop a computational grid in the shape of complex three-dimensional geometries. The program can solve for pressure and velocity, as well as for shear stress, throughout the modeled geometry. This allows comparison of our experimental results with fluid dynamic theory.

(4) A miniaturized compression testing device was constructed using a Starrett precision digital micrometer depth gauge with a 0.25-in diameter barrel. The stents were held to an adjustable immobilizing jig by placing two small wire clips on the bottom surface of the stent ends. This device kept the stents from moving away from the compression barrel and prevented longitudinal elongation of the lower half of the stent surface. To simulate the force of an eccentric lesion of the arterial wall on the test stents, they were incrementally compressed by the barrel of the micrometer to a maximum of 50% of the initial diameter. At each increment of deformation, the resultant force was measured by a Mettler digital pan balance, upon which the stent and jig were placed. Stress-strain data was collected from the force-displacement data.

(5) A glass model of the human aorta was fabricated from anatomical diagrams. The aorta was inserted into a pulsatile flow loop. The distal ends of the various branch arteries were connected with Tygon tubing, the ends of which were arranged in a linear array and positioned over a collecting reservoir. A comparable array of 20-ml vials was arranged in a rack and used to collect the effluent fluid from the tubing as a means of sampling the effluent fluid for injected dye concentration. Several different types of angiographic injection catheters were inserted into the aortic model, just proximal to the first series of branches. Contrast agent colored with red dye was injected at

rates of 10 ml/min, 15 ml/min, and 20 ml/min using a Medrad infusion pump. Samples were analyzed with a spectrophotometer for dye concentration to assess uniformity of contrast distribution in the aortic branches.

MAJOR FINDINGS: (1) Our results indicate that streaming of infused solution can occur in pelvic arteries when infusion rates are low (2 ml/min). The ultimate distribution of infused material into distal arteries is a strong function of catheter position. Reinsertion of the catheter into the model to a nominally identical position in the uterine artery, with a degree of accuracy comparable to that expected in a clinical situation, showed a significantly different pattern of distribution from insertion to insertion. Positioning the catheter in the hypogastric artery, a common location for prior clinical studies, showed that a significant amount of streaming can direct the injectate into the arteries of the rectum and bladder--where they can present undesirable toxic affects--and bypass the uterine artery, which feeds the desired target tissue. Rapid bolus injections from a pulsed infusion pump greatly improves the distal distribution of injectate.

(2) A complete fluid-sheared cell-culture system is in the final stages of calibration and troubleshooting. An endothelial cell line is being prepared for culturing in the parallel-plate chamber.

(3) The experimental portion of the transition-to-turbulence study has lead to an empirical correlation based on two dimensionless groups: α (Wormersley's dimensionless frequency) and a dimensionless stroke volume, St (the Strouhal number). The correlation works well to predict the critical Reynolds number of the peak oscillatory flow at which transition occurs. Laser Doppler data has provided the velocity profiles for laminar, transitional, and turbulent flow, which have been compared to theoretical profiles calculated from Poiseuille's equation, Blasius's equation, and Wormersley's equation. Progress on the numerical analysis stems from the generation of the proper grid in the finite element scheme for calculating fluid mechanical parameters for straight tubes and branched vessels. Further progress in the numerical analysis awaits the imminent arrival of an improved version of the numerical program, FIDAP.

(4) Three different brands of stents have been tested in the novel stress/strain apparatus: Palmaz, Wallstent, and Strecker stents. The moduli of elasticity of the 10-mm stents, as gm/cm², are as follows:

Palmaz = 7410

Wallstent (single-weave) = 1717

Strecker (nitinol) = 353

Wallstent (double-weave) = 4019

Strecker (titanium) = 605

The Palmaz showed a permanent deformation after a 15%-20% radial compression. All other stents showed full elastic recoil, even after radial deformations up to 50%.

(5) Two types of catheters were tested: halo catheters and pigtail catheters. For both types, the homogeneity of delivery improved with increasing injection rate, but this effect was more pronounced for the halo catheter. Overall, the halo catheter gave the most uniform dye delivery, especially at the highest delivery rate (20 ml/sec).

SIGNIFICANCE: (1) To achieve maximum therapeutic benefit with minimum toxicity to the patient, proper delivery of drug solution to the desired tumor-bearing region must be achieved. *In vitro* flow models are very useful in assessing delivery methodologies (e.g., catheter placement, catheter design, and injection procedures). Catheter placement is critical for targeting the desired tissue region for drug delivery. Rapid bolus injections can enhance mixing.

(2) The adhesion of monocytes to arterial endothelial cells is believed to be one possible initiating event in atherosclerosis. Previous studies of monocyte adhesion in synthetic tubes demonstrated the fluid mechanical factors contributing to monocyte adhesion. Further analysis of monocyte interaction with the biologically active surface of endothelial cells is the next logical step to understanding the influence of flow in the atherogenic process. Cultured endothelial cells provide a convenient medium for controlled study of this interaction.

(3) Transition to turbulence in hemodynamics may have a significant role in numerous pathological processes. The ability to predict the presence of turbulence is therefore useful. Experimental correlations offer one method to predict turbulence. Numerical calculations provide another framework for assessing the nature of flow characteristics in complex geometries and are complementary to the experimental work.

(4) The mechanical strength of stents determines their ability to withstand the compressive forces of the arterial wall during the restenosis process, which frequently involves compression by eccentric lesions. Our stress/strain device tests the stents in this eccentric mode. It provides a system for evaluating the strength of various types of stents. The Palmaz demonstrates a greater resistance to initial deformation, but becomes permanently deformed after a 15% radial compression. This degree of compression can occur in femoral artery stents (for example, if the patient sits on a sharp edge or experiences a sharp blow to the leg). The Wallstent and Strecker stents compress more easily, but generally can recoil when the force is removed. Each type of stent may have an appropriate use in certain situations.

(5) The most useful angiographic catheter is one that gives the most uniform distribution of injected contrast agent with the

least amount of contrast. The halo catheter performs better than the pigtail catheter at 20 ml/sec. The *in vitro* model is useful in assessing these catheters.

PUBLICATIONS: Froelich JL, Barth KH, Lutz RJ, Lossef SV, Lindisch D. Injection characteristics and downstream contrast material distribution of flush aortography catheters: *In vitro* study. JVIR 1992;3:713-18.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10327-05 BEI
PERIOD COVERED October 1, 1992 to September 30, 1993		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Mass Mapping of Macromolecular Assemblies		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Richard D. Leapman, Ph.D. Physical Scientist OD, BEIP, NCRR		
COOPERATING UNITS (if any) LN, NINDS (S. B. Andrews, P. E. Gallant, T. S. Reese); LSBR, NIAMS (A. C. Steven); Lehigh University (J. A. Hunt)		
LAB/BRANCH Biomedical Engineering and Instrumentation Program		
SECTION Electron Beam Imaging and Microspectroscopy Group		
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, MD 20892		
TOTAL STAFF YEARS: 0.3	PROFESSIONAL: 0.3	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews ☐ (b) Human tissues ☒ (c) Neither		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The BEIP field-emission scanning transmission electron microscope has been used to obtain low-dose digital annular dark-field mass maps of isolated macromolecular assemblies, including the cytoskeleton and viruses. The technique has been applied to determine how the transport motor molecule, kinesin, mediates microtubule interactions <i>in vitro</i>. Maps of these structures provided information about the shapes and mass distributions of squid brain kinesin and taxol-stabilized microtubules. It was found that kinesins were loosely attached to the carbon support as asymmetrical dumbbell-shaped molecules, 40 to 52 nm long, with a mass of 379 ± 15 kDa. The mass distribution and shape of kinesin suggest that these images represent the molecule in its shortened conformation. Kinesin microtubule complexes were organized as bundles of linearly arrayed microtubules, stitched together at irregular intervals by crossbridges (typically <25 nm long). The crossbridges had a mass similar to that of the isolated single molecule. The results suggest that kinesin has a second microtubule binding site, in addition to the known site on the motor domain of the heavy chain.		

OBJECTIVES: To determine molecular weights of isolated macromolecular assemblies by using low-dose, elastic dark-field imaging in the high-resolution scanning transmission electron microscope (STEM).

METHODS EMPLOYED: Proteins were adsorbed onto thin, 3-nm carbon support films on EM grids by injecting samples into a volatile buffer. After blotting, the grids were plunge-frozen into liquid cryogen and cryotransferred into a VG Microscopes HB501 STEM. Dark-field images were recorded with single-electron sensitivity by means of a PC486-based acquisition system. The images were processed using the IMAGE program running on a Macintosh II computer.

MAJOR FINDINGS: Microtubules without kinesin showed no tendency to self-associate, but when incubated in the presence of purified squid brain kinesin, they associated into large clumps. Kinesin has not previously been characterized by direct observation of the native molecules. STEM images revealed a bilobed structure from which boundaries of structural domains can be estimated. Analysis of the mass distribution between domains led to the conclusion that the end of higher molecular mass is, in fact, the light-chain end of the molecule. Once the size and mass distribution for a single kinesin molecule was established, it was possible to deduce that the crossbridges decorating microtubule bundles were also single kinesins.

SIGNIFICANCE: The STEM observations suggest two possible important roles for non-organelle kinesin: directionally oriented motors to power microtubule sliding along other microtubules, thereby promoting extension of microtubule networks; or dynamic spacers, maintaining and tensioning the microtubule network in coordination with other cytoskeletal components. An appropriate opposition of kinesin-based forces might serve for dynamic stabilization of parallel microtubule arrays. Where microtubules are arranged antiparallel with overlapping plus-ends, as in mitotic spindles, activation of a plus-end-directed motor would lead to spindle elongation. Antiparallel microtubules are found in developing dendrites and axons, so kinesin may also play an important role in the developing brain.

PROPOSED COURSE: It is planned to measure the masses of molecularly engineered kinesins that exhibit different motor functions. Mass mapping will also be applied to neurofilaments and to the study of viruses.

PUBLICATIONS: Andrews SB, Gallant PE, Leapman RD, Reese TS, Schnapp BJ. Single kinesin molecules cross-bridge microtubules *in vitro*. *Proc Natl Acad Sci (USA)* 1993;90:6305-8.

Andrews SB, Leapman RD. Biological scanning transmission electron microscopy. *Microscopy and Analysis (U.K.)* 1993 (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10329-05 BEI
PERIOD COVERED October 1, 1992 to September 30, 1993		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Optimized Mammography Instrument		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Alec Eidsath, Ph.D. Staff Fellow MES, BEIP, NCRR		
COOPERATING UNITS (if any) NCDRH, FDA (R. Jennings); DR, CC (J. Vucich)		
LAB/BRANCH Biomedical Engineering and Instrumentation Program		
SECTION Mechanical Engineering Section		
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, Maryland 20892		
TOTAL STAFF YEARS: 0.2	PROFESSIONAL: 0.2	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) X (a) Human (b) Human (c) Neither subjects tissues (a1) Minors (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Mammography plays a vital part in the early detection of breast cancer. Unfortunately, mammography units being used today have never been analyzed from a systems point of view. Multiparameter optimization studies of a mammography system have been reported by Muntz et al. (Med Phys 12:5, 1985) in which the patient dose was minimized while satisfactory image quality was maintained. The most important new feature of the design is the presence of an array of thin metal vanes arranged in an arc about the x-ray source, between the breast and the film. The vanes absorb scattered radiation and thus improve the quality of the image. In order for the vanes not to image on the film, the grid must move in a precisely controlled profile during the exposure.		

OBJECTIVES: To develop an improved antiscatter device for use in mammography.

METHODS EMPLOYED: Advanced composite materials will be used in the design of parts of the device, thus providing both high strength and low x-ray absorbance. The motion will be controlled using a stepping motor with programmable motion profiles. Sensing of the position of the grid will be accomplished using high-speed fiber-optic sensors.

SIGNIFICANCE: This topic has received a great deal of attention in the press recently, and regular mammograms are now recommended for all women over the age of 40. Since more mammograms will be taken, it is extremely important to reduce the dose to the patient in order to lessen any possible problems caused by radiation. The new instrument has a theoretical dose reduction factor of three.

PROPOSED COURSE: The moving grid assembly has been designed and constructed and has undergone almost a year of transmission of radiation testing at the FDA. The assembly as constructed reduces the radiation dose to the patient by a factor somewhat greater than two. In July 1992 it was sent to Fischer Imaging in Colorado, to be installed on a slightly modified mammography unit. Unfortunately, the grid itself was damaged during the shipment. A new grid was then designed and delivered to Fischer in February 1993. When Fischer has completed the installation, the unit will be returned for patient trials here at the NIH. If the trials are successful, the new instrument will be commercialized by Fischer.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10331-04 BEI												
PERIOD COVERED October 1, 1992 to September 30, 1993														
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Microanalysis of Rapidly Frozen Tissue in the Field-Emission STEM														
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 40%;">Richard Leapman, Ph.D.</td> <td style="width: 30%;">Physical Scientist</td> <td style="width: 30%;">OD, BEIP, NCRR</td> </tr> <tr> <td>Shanling Shi, M.S.</td> <td>Visiting Associate</td> <td>OD, BEIP, NCRR</td> </tr> <tr> <td>Stanley Sun, Ph.D.</td> <td>Visiting Fellow</td> <td>OD, BEIP, NCRR</td> </tr> <tr> <td>Carol Swyt, M.S.</td> <td>Physical Scientist</td> <td>OD, BEIP, NCRR</td> </tr> </table>			Richard Leapman, Ph.D.	Physical Scientist	OD, BEIP, NCRR	Shanling Shi, M.S.	Visiting Associate	OD, BEIP, NCRR	Stanley Sun, Ph.D.	Visiting Fellow	OD, BEIP, NCRR	Carol Swyt, M.S.	Physical Scientist	OD, BEIP, NCRR
Richard Leapman, Ph.D.	Physical Scientist	OD, BEIP, NCRR												
Shanling Shi, M.S.	Visiting Associate	OD, BEIP, NCRR												
Stanley Sun, Ph.D.	Visiting Fellow	OD, BEIP, NCRR												
Carol Swyt, M.S.	Physical Scientist	OD, BEIP, NCRR												
COOPERATING UNITS (if any) LN, NINDS (S. B. Andrews, T. S. Reese); LMMB, NIAAA (R. Veech); LPD, NIAID (T. E. Nash); Arkansas State University (R. A. Buchanan); Case Western Reserve University (D. D. Landis)														
LAB/BRANCH Biomedical Engineering and Instrumentation Program														
SECTION Electron Beam Imaging and Microspectroscopy Group														
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, MD 20892														
TOTAL STAFF YEARS: 1.8	PROFESSIONAL: 1.8	OTHER: 0.0												
CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%; vertical-align: top;"> ☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews </td> <td style="width: 33%; vertical-align: top;"> ☐ (b) Human tissues </td> <td style="width: 33%; vertical-align: top;"> ☒ (c) Neither </td> </tr> </table>			☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews	☐ (b) Human tissues	☒ (c) Neither									
☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews	☐ (b) Human tissues	☒ (c) Neither												
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Freeze-dried, ultrathin cryosections of directly frozen mouse liver and brain have been prepared and characterized by low-dose dark-field scanning transmission electron microscopy (STEM). These improved cryosections gave images comparable to those from conventional plastic sections. They were thin enough to use established dark-field techniques (modified for thickness-dependent nonlinearities) in order to measure the dry mass fraction of individual organelles, and hence to deduce their water content. Digital STEM imaging, in combination with electron and x-ray spectroscopy, has important biological applications, as illustrated by studies on calcium regulation in Purkinje neurons. Calcium concentrations per unit dry weight of dendritic compartments were determined by the peak/continuum method of energy-dispersive x-ray spectroscopy (EDXS), which necessarily overstates elemental concentrations because of beam-induced mass loss. The dry-mass content of organelles at low dose and the percent of dry mass retained after analysis at high dose were used to correct elemental concentrations for mass loss. Results indicated that the major calcium storage organelle in Purkinje cell dendrites is the endoplasmic reticulum, of which there are two types, distinguished by their levels of calcium. These techniques are being applied to characterize elemental and ionic distributions in other tissues, including hepatocytes and the protozoan parasite <i>Giardia</i> .														

OBJECTIVES: To determine changes that occur in ionic composition of postsynaptic terminals in cerebellar cortex during neuronal activity. Of particular interest is the composition of membrane-bound structures of endoplasmic reticulum, which are believed to regulate calcium during synaptic activity.

METHODS EMPLOYED: Dark-field STEM micrographs were recorded digitally with a PC486-based acquisition system that provided nearly single-electron sensitivity. Extremely low probe currents of order 1 pA were used to produce a low-radiation exposure on the specimen and to prevent saturation of the detector. Dark-field data were processed and analyzed on an Apple Macintosh II computer. Mass loss was calculated as the ratio of relative mass of organelles following high-dose exposure to the mass of the same organelles before exposure. Energy-dispersive x-ray spectra were acquired with an ultrathin window detector and were transferred to the Macintosh for analysis by the DeskTop Spectrum Analyzer program.

MAJOR FINDINGS: By combining state-of-the-art cryopreparation techniques with low-dose dark-field imaging in the field-emission STEM, it has been possible for the first time to attain structural quality in cryosectioned tissue that is comparable to conventional thin sections. Moreover, the sections are thin enough—below one inelastic mean free path when frozen-hydrated and substantially thinner after freeze-drying—to analyze quantitatively in the electron microscope, so that established dark-field techniques for mass analysis can be used to measure the mass of individual structures and organelles *in situ*. The results suggest several new biological applications for such cryosections, which we have demonstrated in the context of understanding synaptic activity-dependent calcium regulation in Purkinje cells of the mouse cerebellar cortex. Specifically, we have determined the following applications: 1) the organization of unfixed, directly frozen parallel fiber/Purkinje cell synapses and Purkinje cell dendrites and dendritic spines in comparison with corresponding conventional preparations; 2) the *in situ* masses of undamaged dendritic organelles and compartments—endoplasmic reticulum, mitochondria, and cytoplasm—that are thought to be involved in calcium regulation; 3) the effects of electron-beam damage on the structure and masses of these organelles; and 4) the elemental content of these organelles, corrected for the damaging effects of analytical electron doses. Elemental analysis demonstrates that, as previously found for a variety of cell types, the endoplasmic reticulum is the major calcium storage organelle in Purkinje cell dendritic shafts.

SIGNIFICANCE: Our results imply that low-dose structural imaging and mass analysis of such sections in combination with x-ray and electron spectroscopy is a promising approach to the quantitation of the changes in mass, mass distribution, and elemental composition that often underlie organelle function. This, in turn, offers the possibility of directly studying *in situ* subcellular physiology.

PROPOSED COURSE: Further development of STEM imaging techniques will be implemented, with the aim of automating the acquisition and analysis of the digital micrographs. The methods will be applied to various biological systems.

PUBLICATIONS: Buchanan RA, Leapman RD, O'Connell MF, Reese TS, Andrews SB. Applications of the field-emission STEM to subcellular structure and analysis of ultrathin tissue cryosections. J Struct Biol 1993 (in press).

Andrews SB, Buchanan RA, Leapman RD. Characterization of calcium-release organelles in Purkinje cells by combined x-ray and mass analysis. Proceedings of the 27th annual meeting of the Microbeam Analysis Society. San Francisco: San Francisco Press, 1993 (in press).

Andrews SB, Buchanan RB, Leapman RD. Characterization of cryosections in the FE-STEM: specimen damage during freeze-drying and analysis. Proceedings of the 27th annual meeting of the Microbeam Analysis Society. San Francisco: San Francisco Press, 1993 (in press).

Leapman RD, Sun S, Shi S, Buchanan RA, Andrews SB. STEM measurement of subcellular water distributions. Proceedings of the 51st annual meeting of the Electron Microscopy Society of America. San Francisco: San Francisco Press, 1993 (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10337-04 BEI									
PERIOD COVERED October 1, 1992 to September 30, 1993											
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Nuclear Medical Imaging: Scintigraphic Imaging System for Small Animals											
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">Allen Markowitz, M.S.</td> <td style="width: 33%;">Electrical Engineer</td> <td style="width: 33%;">ACES, BEIP, NCRR</td> </tr> <tr> <td>Thomas Tedder</td> <td>Instrument Maker</td> <td>BEIP, NCRR</td> </tr> <tr> <td>Burton Chidakel, B.S.</td> <td>Instrument Maker</td> <td>BEIP, NCRR</td> </tr> </table>			Allen Markowitz, M.S.	Electrical Engineer	ACES, BEIP, NCRR	Thomas Tedder	Instrument Maker	BEIP, NCRR	Burton Chidakel, B.S.	Instrument Maker	BEIP, NCRR
Allen Markowitz, M.S.	Electrical Engineer	ACES, BEIP, NCRR									
Thomas Tedder	Instrument Maker	BEIP, NCRR									
Burton Chidakel, B.S.	Instrument Maker	BEIP, NCRR									
COOPERATING UNITS (If any) NM, CC (M. Green)											
LAB/BRANCH Biomedical Engineering and Instrumentation Program											
SECTION Applied Clinical Engineering Section, CCIS											
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, Maryland 20892											
TOTAL STAFF YEARS: 0.7	PROFESSIONAL: 0.4	OTHER: 0.3									
CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%; vertical-align: top;"> ☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews </td> <td style="width: 33%; vertical-align: top;"> ☐ (b) Human tissues </td> <td style="width: 33%; vertical-align: top;"> ☒ (c) Neither </td> </tr> </table>			☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews	☐ (b) Human tissues	☒ (c) Neither						
☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews	☐ (b) Human tissues	☒ (c) Neither									
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) A prototype analog scintigraphic imaging system has been developed for use in small animal imaging studies. Unlike conventional scintillation cameras, this system uses a single position-sensitive photomultiplier tube (PMT) to determine the location of scintillation events within the sodium iodide scintillator. Performance measurements and imaging studies in small animals confirm the ability of this system to obtain high-resolution images of the lungs, heart, brain, and other structures in animals the size of rats and guinea pigs. The camera is coupled to a commercial software/hardware package, running on a Macintosh computer system that permits all common forms of nuclear medicine acquisition/processing procedures routinely utilized in human studies to be performed on small animals. The instrument developed is a low-cost, low-linearity system. Work during this project period has focused on an evaluation of a low-cost, special-purpose, improved linearity system. At present, this proposed system will input a linear pulse to a flash or Sigma-Delta ADC. Position and energy information will then be input to either a DSP board within an embedded computer, into an EPROM/FPGA logic design, or to a stand-alone computer. The optimized output from this system will then be directed to the Macintosh through a video DAC.											

OBJECTIVES: Creation of a small-field-of-view scintigraphic imaging system capable of all forms of nuclear tracer imaging (e.g., planar projection imaging, R-wave or stimulus-gated imaging, time-sequence imaging, etc.) in small animals.

METHODS EMPLOYED: A scintigraphic imaging system has been developed that uses analog custom-designed nuclear instrumentation modules (NIMs). These modules compute event position location and corrected event energy. Pulse-height is determined with commercially available electronic NIMs. The positions of valid events are then input to a Macintosh acquisition system. An animal rotation device was added to the system to convert the system to a single photon-emission computerized tomography (SPECT) imaging system for small animals. Over 30 custom NIM modules were designed. The modules included a trigonometric function converter, XY+Z multiplier, 3-input summer and inverter, dual square root, position computer interface, dual divider, dual attenuator amplifier, hex voltage variable power supply, and angular position.

SIGNIFICANCE: Successful completion of this project will result in a very flexible, relatively low-cost nuclear tracer imaging system, capable of yielding high-resolution images of single photon-emitting radiopharmaceuticals anywhere in the bodies of small animals, such as the monkey. In particular, this system will permit novel tracer methods and new radiopharmaceuticals for studying the heart and brain to be evaluated and screened before potential application to man. As a by-product of this work, the experience gained in the use of the Macintosh II data acquisition/processing system should allow evaluation of this system's suitability for general use with human patients. Similarly, evaluation of the position-sensitive phototube/cameras should suggest other applications for these devices.

PROPOSED COURSE: System characteristics and requirements are being defined for the new imaging system. Upon completion of this task, the low-cost, high-resolution, high-speed system will be evaluated. If it is competitive with other approaches, it will be fabricated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10339-04 BEI
PERIOD COVERED October 1, 1992 to September 30, 1993		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Support of Diagnostic Radiology Research Program		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation) Ronald L. Levin, Sc.D. Biomedical Engineer MES, BEIP, NCRR		
COOPERATING UNITS (If any) DSB, DCRT (M. A. Douglas); LDRR, OIR, OD (J. A. Frank)		
LAB/BRANCH Biomedical Engineering and Instrumentation Program		
SECTION Mechanical Engineering Section		
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, Maryland 20892		
TOTAL STAFF YEARS: 0.9	PROFESSIONAL: 0.9	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews ☐ (b) Human tissues ☒ (c) Neither		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The NIH has been requested by Congress to expand its research to include aspects of radiology and imaging. To this end, a Laboratory of Diagnostic Radiology Research has been established. This program is designed to give radiologists, as well as others from the American radiological community, training in research methodology and technique. Part of that training shall include performing research with NIH staff scientists and clinicians on the latest techniques for the following procedures: (1) whole-body magnetic resonance instrumentation, and (2) the visualization and analysis in multiple dimensions of multimodality medical images. Additional NMR instrumentation and image processing hardware and software have therefore been acquired. The BEIP is providing logistical and scientific expertise in support of the program.		

OBJECTIVES: (1) To facilitate magnetic resonance research in the fields of imaging, spectroscopy, angiography, perfusion, and diffusion. (2) To facilitate the exchange of medically related images by permitting images from multiple sources to be accessed via a single archive and in a single file format, and to facilitate the manipulation and comparison of medically related images together through the use of a specially designed biomedical workstation.

PROPOSED COURSE: (1) A Signa 1.5-T whole-body magnetic resonance unit being used by this program is continually being upgraded so that it remains at the state of the art. Recent upgrades include the migration from 4.7 to 5.X hardware/software revision level and the addition of specialized gradient and RF coils to facilitate functional imaging.

(2) A multidimensional radiological image processing system (MRIPS), the primary use of which will be the visualization and analysis of multimodality medical images, is being deployed. The system consists of a combination of computer software and hardware suitable for the development of new image visualization and image analysis tools, as well as for the use of existing image processing software packages.

The MRIPS will consist of the following hardware and software: (1) a network of image visualization and image analysis workstations, supported by four centrally located data servers containing over 100 Gigabytes of data; (2) the UNIX operating system; (3) an X11/Motif graphic user interface; (4) software tools for the development of new image visualization and image analysis programs; (5) an image analysis application; and (6) network and system software compatible with existing NIH computer networks and image processing programs.

The MRIPS will be used by trainees associated with the LDRR and by other NIH scientists for the analysis of medical images obtained by computerized tomography (CT), magnetic resonance imaging (MRI), magnetic resonance spectroscopy (MRS), positron emission tomography (PET), etc. Many of the studies will involve determination of the relationships between anatomical and physiological image data obtained from various body parts. Of particular importance in this regard is the need to obtain spatial registration of MRIPS-collected data both efficiently and accurately.

METHODS EMPLOYED: This system, the Multimodality Radiology Image Processing System (MRIPS), consists of the following components:

- a. AFS (Andrew File System) -based data/computation servers consisting of three DECstation 5000/200 CPUs and one SUN SPARCstation 2 CPU with over 100 Gbyte of magnetic disk space; one SUN SPARCstation 2 AFS/NFS gateway; one DECstation

5000/125 AFS-client; one SUN ELC tape-backup system; associated networking and communication subsystems; and other miscellaneous peripherals.

- b. Numerous AFS-based and NFS-based workstations consisting of SUN SPARCstations and superSPARCstations, MIPS-based and ALPHA-based DECstation, HP Series 700 machines, and SGI workstations.
- c. Special dataset transfer, format conversion, and data management routines, including Kerberos-based security mechanisms and a dataset registry.
- d. The **MEDx** image processing software package, consisting of special medically related image processing enhancements to Sterling, Virginia-based BDS Systems' commercially available nonmedical **XcaliburTM** image processing package.

NIH currently has licenses to use **XcaliburTM** on over 100 workstations, as well as on the data/computation servers. There is a large demand from members of the intramural NIH community for additional licenses to use **MEDx** on existing workstations, in addition to the need to acquire additional workstations for those individuals/laboratories who need to do medical image processing but who do not currently have appropriate UNIX-based workstations. There is also a demand for enhancements to be made to MRIPS, in addition to long-term support and training.

RECENT ACCOMPLISHMENTS: During the past year, MRIPS has gone from an unofficial, amorphous entity to a formal section within LDRR with its own budget and steering committee. Dr. Ronald Levin of BEIP has been appointed Chief of MRIPS, with a joint appointment within LDRR/OIR/OD. Ms. Margaret Douglas of DCRT has been appointed Deputy Chief of MRIPS, with a joint appointment within LDRR/OIR/OD. The mission of this section is to maintain and enhance MRIPS as an intramural NIH research resource in medical image processing. This responsibility will include performing all future MRIPS software and hardware day-to-day maintenance operations and upgrades in support of collaborative research among NIH's numerous ICDs. Additional resources, as needed, will be drawn from the Advanced Laboratory Workstation (ALW) group and the Computer Systems Branch (CSB) of DCRT for Andrew File System (AFS) support; the Network Systems Branch (NSB) of DCRT and the Scientific Equipment Services (SES) portion of BEIP/NCRR for networking support; and the Office of the Director of BEIP/NCRR and the Distributed Systems Branch (DSB) of DCRT for image processing support.

In order to ensure that the services provided by the MRIPS Section of LDRR represent those desired by NIH ICDs, a steering committee has been established to provide guidance for MRIPS' operations and developments. This committee meets at least once a month, and is

responsible for essentially the same technical aspects of MRIPS that the steering committee for the *In Vivo* NMR Research Center currently assumes for that NCRR administrative resource--namely, the establishment of priorities, time lines, etc. Representatives are drawn from most of NIH's ICDs.

PUBLICATIONS: Levin RL, Douglas MA, Frank JA, Gladish R. Multimodality Radiological Image Processing System. In: Robb RA, ed. Visualization in biomedical computing 1992. New York: SPIE, 1992;487-94.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10342-04 BEI
PERIOD COVERED October 1, 1992 to September 30, 1993		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Computer-Based, Dual-Pump HPLC Driver System		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) John W. Cole, MSEE Electrical Engineer EEES, BEIP, NCRR		
COOPERATING UNITS (if any) CE, NIDDK (R. Lippoldt)		
LAB/BRANCH Biomedical Engineering and Instrumentation Program		
SECTION Electrical and Electronic Engineering Section		
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, Maryland 20892		
TOTAL STAFF YEARS: 0.2	PROFESSIONAL: 0.2	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews ☐ (b) Human tissues ☒ (c) Neither		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) High-Performance Liquid Chromatography (HPLC) is a common method used to separate various molecules on the basis of their size. The solution to be analyzed is passed through a long column packed with tiny beads. Larger molecules tend to have a more difficult time passing through the column, while smaller molecules move more rapidly, leading to the desired size-separation effect. By forcing the solution through the column under high pressure, chromatographic separations are achieved much faster and with greater resolution, compared to standard chromatographic methodologies. In most typical HPLC systems, a single pump is used to drive the solution to be analyzed through the column. Recently interest has been shown in using a dual-pump driver system. A system of this type would allow the reaction and by-products of two chemical reactants to be studied and separated as the reaction occurs and passes through the column.		

OBJECTIVES: To develop a computer-based, dual-HPLC-pump driver system that allows for linear, upward-curved, and downward-curved mixing gradients. The system must also allow for complete control of the following experimental parameters: total run time, total volume of liquid pumped, gradient type (linear or curved), gradient deviation or amount of curvature, and the number of different straight-line approximations used to make up a curve.

METHODS EMPLOYED: A Metrabyte counter timer board (CTM-5) was installed in an IBM-AT-compatible personal computer. The CTM-5 control software was interfaced with a custom program, written in MS-Quick Basic, to instruct the CTM-5 board to produce the required signals necessary to control the pumps.

SIGNIFICANCE: A computer-based, dual-pump HPLC system will allow various new experiments to be performed, while obtaining the benefits of computer-guided accuracy. Experiments may be repeated easily and accurately for experimental result verification.

PROPOSED COURSE: The IBM-AT computer system was completed in mid-1990. Over the last year, an identical system has been configured, centered around an IBM-PS2 computer. A PS2-compatible CTM-05 Metrabyte counter timer board was installed, as well as a modified copy of the pump driver software. Both the AT and PS2 computer-based systems are now in operation. Modifications will occur as required.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10343-04 BEI									
PERIOD COVERED October 1, 1992 to September 30, 1993											
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Polynomial Spline Signal Processing Techniques											
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 40%;">Michael Unser, Ph.D.</td> <td style="width: 30%;">Visiting Scientist</td> <td style="width: 30%;">OD, BEIP, NCRR</td> </tr> <tr> <td>Akram Aldroubi, Ph.D.</td> <td>Staff Fellow</td> <td>OD, BEIP, NCRR</td> </tr> <tr> <td>Murray Eden, Ph.D.</td> <td>Director</td> <td>OD, BEIP, NCRR</td> </tr> </table>			Michael Unser, Ph.D.	Visiting Scientist	OD, BEIP, NCRR	Akram Aldroubi, Ph.D.	Staff Fellow	OD, BEIP, NCRR	Murray Eden, Ph.D.	Director	OD, BEIP, NCRR
Michael Unser, Ph.D.	Visiting Scientist	OD, BEIP, NCRR									
Akram Aldroubi, Ph.D.	Staff Fellow	OD, BEIP, NCRR									
Murray Eden, Ph.D.	Director	OD, BEIP, NCRR									
COOPERATING UNITS (if any) None											
LAB/BRANCH Biomedical Engineering and Instrumentation Program											
SECTION Office of the Director											
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, MD 20892											
TOTAL STAFF YEARS: 0.5	PROFESSIONAL: 0.5	OTHER: 0.0									
CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">_ (a) Human subjects</td> <td style="width: 33%;">_ (b) Human tissues</td> <td style="width: 33%;">X (c) Neither</td> </tr> <tr> <td>_ (a1) Minors</td> <td></td> <td></td> </tr> <tr> <td>_ (a2) Interviews</td> <td></td> <td></td> </tr> </table>			_ (a) Human subjects	_ (b) Human tissues	X (c) Neither	_ (a1) Minors			_ (a2) Interviews		
_ (a) Human subjects	_ (b) Human tissues	X (c) Neither									
_ (a1) Minors											
_ (a2) Interviews											
SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.) Conventional digital signal processing techniques consider signals that are represented by a set of uniformly spaced sample values. Although most processing algorithms are derived within a purely discrete framework, there are a variety of biomedical problems (e.g., detection of anatomical structures in images, registration) that would be better formulated by considering a signal as a continuous real-valued function defined over some domain. This study is concerned with the development of new processing techniques that represent signals by continuous polynomial spline functions. Traditionally, polynomial spline interpolation or approximation problems are approached using a matrix formulation. Our initial contribution has been to derive efficient computational algorithms using recursive digital filters. These techniques have been applied to the design of fast algorithms for image interpolation and compression. We have also derived a sampling theory for polynomial splines that generalizes Shannon's sampling theorem for bandlimited functions. In addition, we have defined a whole family of polynomial spline wavelet transforms that allows the expansion of continuous image functions in terms of basis functions obtained by dilatation and expansion of a single prototype. Such wavelet transforms may provide space/frequency signal decompositions that are close to optimal, according to the uncertainty principle.											

OBJECTIVE: To develop new processing techniques using continuous polynomial spline representations of signals. These techniques can be useful for resolution conversions (zooming and shrinking), data compression, multiscale signal analyses, edge detection, or signal differentiation.

METHODS EMPLOYED: The whole approach is based on the premise that any polynomial spline function can be represented as a weighted sum of shifted B-spline basis functions. A signal is therefore entirely characterized by the discrete sequence of its B-spline coefficients, which can be computed efficiently by digital filtering. These algorithms have been coded in FORTRAN on a low-end biomedical image processing workstation based on a standard Apple Macintosh II.

MAJOR FINDINGS: We have derived several theoretical results characterizing the performance of polynomial spline representations as the order of the splines tends to infinity:

(1) A B-spline interpolation is asymptotically equivalent to an ideal lowpass filter (classical sinc interpolator).

(2) The optimal prefilter for B-spline approximation asymptotically tends to an ideal lowpass filter (classical anti-aliasing filter).

(3) The B-spline wavelets asymptotically tend to a modulated Gaussian (Gabor function). The B-spline wavelet transform is therefore asymptotically optimal in terms of its time-frequency localization (uncertainty principle).

Properties (1) and (2) indicate that polynomial spline approximation can be viewed as a generalization of the classic sampling approach for bandlimited signals dictated by Shannon's sampling theorem. Property (3) provides the connection with the Gabor transform, which is a classic method for time/frequency signal analysis.

SIGNIFICANCE: To provide NIH scientists with new tools for the processing of biomedical signals. To increase the efficiency of resolution conversion and display algorithms on image processing workstations.

PROPOSED COURSE: We will continue our investigation of polynomial spline pyramids and wavelet decompositions for multi-resolution signal analysis. We have also started developing a spline-based multiscale registration algorithm for the alignment of brain slices for 3-D reconstruction.

PUBLICATIONS: Aldroubi A, Unser M, Eden M. Cardinal spline filters: stability and convergence to the ideal sinc interpolator. Signal Processing 1992;28(2):127-38.

Unser M, Aldroubi A. Spline multiresolutions and wavelet transforms. In: Proceedings of the IEEE-SP International Symposium on Time-Frequency and Time-Scale Analysis. Victoria, British Columbia: IEEE, 1992:315-18.

Unser M, Aldroubi A, Eden M. A family of polynomial spline wavelet transforms. Signal Processing 1993;30(2):141-62.

Unser M, Aldroubi A, Eden M. B-spline signal processing. Part I: theory. IEEE Trans Signal Processing 1993;41(2):821-33.

Unser M, Aldroubi A, Eden M. B-spline signal processing. Part II: efficient design and applications. IEEE Trans Signal Processing 1993;41(2):834-48.

Unser M, Aldroubi A, Eden M. The L_2 polynomial spline pyramid. IEEE Trans Pattern Analysis Mach Intell 1993;15(4):364-79.

Unser M, Eden M. Optimal FIR approximations of inverse filters and perfect reconstruction filter banks. Signal Processing (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10353-03 BEI
PERIOD COVERED October 1, 1992 to September 30, 1993		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Drug Transport in Brain		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Paul F. Morrison, Ph.D. Physical Scientist CHES, BEIP, NCRR Peter J. Basser, Ph.D. Mechanical Engineer MES, BEIP, NCRR Robert L. Dedrick, Ph.D. Chief CHES, BEIP, NCRR		
COOPERATING UNITS (if any) SNB, NINDS (D. Laske, D. Lieberman, K. Bankiewicz, E. Oldfield)		
LAB/BRANCH Biomedical Engineering and Instrumentation Program		
SECTION Chemical Engineering Section		
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, MD 20892		
TOTAL STAFF YEARS: 0.4	PROFESSIONAL: 0.4	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) _(a) Human _(b) Human X (c) Neither subjects tissues _(a1) Minors _(a2) Interviews		
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.) Theoretical transport models of high-flow microinfusion into brain tissue have been developed, treating the brain as either a rigid or a deformable elastic medium into which macromolecular solutions are infused at flow rates from 0.1 to 6.0 μ l/min. Previous work has shown that this technique allows slowly degraded macromolecules to access treatment volumes in the range of at least 14 to 27 cu cm. These computations have been extended to describe more reactive molecules, such as neuropeptides and growth factors. Penetration depths and associated treatment volumes based on pharmacodynamic effect have been calculated and shown to be strongly dependent on metabolic rate. Treatment volumes are expected, respectively, to be 2.7 cu cm (1.7 cm dia.) and 0.5 cu cm (0.98 cm dia.) for substances of 1-hr (e.g., a chimeric neuropeptide) and 10-min (e.g., a growth factor) reaction times. Such substances achieve infusion steady-state concentration profiles over 0.5-hr to 3-hr infusions and, unlike unreactive macromolecules, possess sigmoidal profiles that lack a precipitous front. Although the greater reactivity of these agents reduces expected tissue penetration, it does not alter the relative advantage that high-flow microinfusion of these agents provides over competing low-flow or polymer-dissolution methods. In other experimental work, we have confirmed the prediction that the slowly clearing macromolecule albumin is transported spherically outward in grey matter with a moving front profile until it encounters a grey-white boundary.		

RELEVANT BEIP PROJECTS: 84-125, Drug Transport in Brain.

OBJECTIVES: To understand the fundamental processes involved in the transport of small- and macromolecular-sized molecules in the white and grey matter of the brain. To use this information to predict the distribution of these molecules following various administration protocols, and to assess the role of transport in determining therapeutic or diagnostic outcome.

METHODS EMPLOYED: Both theoretical and experimental methods have been employed. On the theoretical side, transport models were constructed from the differential representations of Darcy flow, diffusion-bulk flow, and continuity applied to a homogeneous, rigid, porous model of the brain. Binding, biochemical reaction, and capillary pore bulk flow and permeation were also included as appropriate sink terms. Peclet numbers for macromolecules allowed diffusional contributions to be ignored for distances up to about 1.5 cm from the catheter tip. Penetration depths at the end of infusion and times of approach to these depths were estimated from simple equations derived from the bulk flow limit of transport. Pharmacodynamic penetration distances were determined by identifying the distance from the catheter tip at which each infusion yielded a concentration or area-under-the-curve value that fell below a specified level. These distances accounted for postinfusion diffusional relaxation, in addition to the initial spread of solute by bulk flow. Experimentally and in collaboration with Dr. Lieberman of Surgical Neurology, ^{14}C -labelled albumin was infused via 170- μ I.D. silica catheters into the caudate nucleus of anesthetized rats in volumes up to 20 μl and at flowrates of 0.05 $\mu\text{l}/\text{min}$. Flowrates lower than those previously used were employed in order to avoid significant pressure deformation. Brains were removed and frozen, and the entire caudate was cut into 20- μ sections and analyzed by quantitative autoradiography.

MAJOR FINDINGS: Earlier work focused on the penetration depths of relatively unreactive macromolecules of 180 kD, in which treatment volumes (based on pharmacodynamic penetration depths) of 14 to 27 cm^3 could be expected in homogeneous brain tissue. Extension of these computations have now been made to smaller, more reactive molecules, so that treatment depths may now be estimated for molecules with characteristic reaction times ranging from 10 minutes to infinity. Treatment volumes are expected to decline to 2.7 cm^3 (1.7 cm diameter) for a substance of 1-hr degradation time (in the range of certain neuropeptides), and to 0.5 cm^3 (0.98 cm diameter) for a substance of only 10-min degradation time (more typical of growth factors). Furthermore, because these more reactive molecules more rapidly approach their end-of-infusion steady-state concentration profiles, these limiting distributions are easily obtained in 0.5-hr to 3-hr infusions. Dosing, however, will be less uniform with radial distance from the catheter tip than with the flat concentration profile expected for unreactive macromolecules administered over 6 to 12 hours.

Experimental work has confirmed the prediction for grey matter that large, unreactive macromolecules which slowly permeate capillaries are characterized by flat concentration profiles that decline precipitously near the location of the solvent front. Profiles for albumin administered by high-flow microinfusion show precipitous declines occurring within a distance of 100 μ in the A-P direction. Distribution of infused solute remains relatively spherical in grey matter until infused volumes become sufficiently large to deliver agent to grey-white interfaces, with subsequent flow there directed along the planes between the two tissue types.

In white matter, review of the relative tissue concentrations of transferrin generated at high flow (4 μ l/min) has suggested that some water filtration may be occurring within a few hundred microns of the catheter tip. Relative concentrations neared unity in this range, and expansion of the interstitial space per se could only account for part of this phenomenon. Because of the highly nonlinear relationship between interstitial hydraulic conductivity and pressure, the high pressures near the tip can be expected to extend farther into the tissue than was previously calculated for lower flow rates. Hence, sufficient elevation of this pressure may be present near the tip to drive some water loss and to concentrate the infusion solute in a volume increment before it moves further out into the tissue. No evidence could be found for filtration continuing much beyond this distance. In addition, no significant role for transferrin binding was found. Infused transferrin concentrations were nearly 5 logs in excess of reported tissue-averaged brain transferrin-receptor densities of 0.04 nM. Using maximal rates of endocytosis measured in teratocarcinoma cells, a maximal uptake rate was calculated that could lead to a concentration reduction of only 0.015% near the leading edge of the infusion profile.

SIGNIFICANCE: Delivery of slowly reacting macromolecules to large volumes of brain tissue suggests that high-flow microinfusion can find application in cancer therapy and gene therapy. Competing osmotic minipump and dissolving pellet technologies are hampered by their inability to dose large volumes of tissue unless many such devices are employed simultaneously. Microinfusion offers greater control than other technologies. Flowrates can be altered rapidly, unlike dissolution rates from implants; surgical intervention can be minimized; in white matter, the normal interstitial fluid flows of about 10 μ /min can be overcome by the infusion-pump flow, to a limit of about 1 cm; high concentrations near the source (potentially toxic), needed to create a high-mass flux in low-flow or pellet technologies, can be avoided in high-flow microinfusion because of the much flatter concentration profile achieved with this technology.

PROPOSED COURSE: Further quantification of macromolecular spread in grey matter is planned. Catheter-tip pressures will be monitored during slow infusions of about 0.02 μ l/min into grey matter in order to verify slow tissue compliance, and will be used in conjunction with the rigid, porous model to estimate the

unsupported hydraulic conductivity for this tissue. SPECT studies are planned in order to examine serial solute distributions and assess the importance of retardation effects in the interstitium. In the case of smaller reactive molecules, future studies will focus on specific compounds, since treatment volumes will be intimately related to their particular biochemistry and to the requirements of the deficiency syndrome or neurodegenerative disorder against which these agents are likely to be targeted. In the long term, the brain-transport model could evolve into a finite element form, capable of accounting for transport in large regions of the brain with anatomically correct regional boundaries and anisotropic flows along fiber tracts.

PUBLICATIONS: Bassar PJ. Interstitial pressure, volume, and flow during infusion into brain tissue. Microvas Res 1992;44:143-65.

Morrison PF, Laske DW, Bobo RH, Oldfield EH, Dedrick RL. High-flow microinfusion: tissue penetration and pharmacodynamics. Am J Physiol 1993 (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 RR 10358-03 BEI

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Heat Capacity Effects in Lipids During Unilamellar/Multilamellar Phase Changes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Courtney P. Mudd, Ph.D. Biomedical Engineer ACES, BEIP, NCRR
Thomas R. Clem, Sr., BSEE Electronics Engineer EEES, BEIP, NCRR

COOPERATING UNITS (if any)

LPB, NIAMS (N. Gershfeld); IR CH, NHLBI (R. Berger)

LAB/BRANCH

Biomedical Engineering and Instrumentation Program

SECTION

Applied Clinical Engineering Section

INSTITUTE AND LOCATION

NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

0.2

PROFESSIONAL:

0.2

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human ☐ (b) Human ☒ (c) Neither
subjects tissues
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Recent studies of the properties of phospholipid dispersions in water indicate that a higher-order phase transition occurs involving a spontaneous transformation from a unilamellar liquid-crystal state to a suspension of large, unilamellar vesicles upon increasing the ambient temperature. It has been suggested that the unilamellar vesicles that form are a critical state. The thermodynamic properties of this transformation have been inferred primarily from the properties of air-water surface films in equilibrium with the dispersed phospholipid phase. A more direct and conceptually simpler analysis of the thermodynamic properties of this higher-order transition may be attained by measurements of the temperature dependence of the heat capacity of the lipid dispersions. Since transformations of this type are believed to be intimately involved in the assembly of cell membranes, we have developed an extremely sensitive differential heat-conduction calorimeter for measuring heat capacities of aqueous membrane lipid dispersions. (Attempts to measure this transformation in commercial calorimeters have not been successful.) This instrument has certain obvious advantages over the commercial differential scanning calorimeters: reproducibility of loading, ± 400 micro-J/deg-cm³; baseline stability, ± 10 micro-J/deg-cm³ per 36 hrs; resolution (± 1 S.D.), ± 50 micro-J/deg-cm³; sample size, 600 microliters. Measurements on DMPC have confirmed that there is an abrupt change in the heat capacity at the transition temperature, T*, and that the transition temperature identified by the heat-capacity change agrees with values determined by other methods.

OBJECTIVE: To use a newly designed differential (two-cell) batch-type heat-conduction microcalorimeter to step through a set of temperatures, including the critical temperature, T^* , and to measure the heat capacity of the lipid sample. If we introduce small, short heat bursts into the cell with the lipid solution, the resulting impulse response will depend upon the thermal properties (thermal conductivity and heat capacity) of the lipid. We developed an R-C model of the calorimeter and used a commercial SPICE (circuit simulation) program to evaluate the calorimeter's performance. We found that with this particular calorimeter design, when the heat burst is applied in the interval of 20 to 30 sec, the pulse response after 40 sec is relatively independent of the thermal conductivity. Since the peak of the differential response occurs at 43 sec, we can use that value as a measure of only the heat capacity of the sample. This feature eliminates the need to make two separate measurements (i.e., thermal conductivity and heat capacity). A custom-designed, 80286-based, digital temperature controller has been interfaced to the 80286-type microcomputer that now handles all data acquisition, analysis, and storage. This feature allows automatic temperature scanning.

SIGNIFICANCE: Equilibrium surface film studies of DMPC (lipid) dispersions have shown that at the critical bilayer temperature, T^* , a unilamellar state is formed from liposomes. We have now shown that there is also a heat-capacity change at this critical temperature. Calorimetry can thus provide a direct measurement of the work of bilayer adhesion, as well as verification of the value of the critical temperature.

PROPOSED COURSE: Future work will involve the calibration and measurement of DMPC properties in D_2O to corroborate other measurements made on DMPC in D_2O ; an investigation of the dynamic nature of the transition at T^* , especially the abnormally long equilibration time for the transition; and the development of a slightly less sensitive but faster scanning version, which can be used mainly to identify the transition temperatures of a wide variety of lipids. (After identification, the behavior around T^* could be investigated in detail with the slower but more sensitive version of the calorimeter.)

PUBLICATIONS: Mudd CP, Gershfeld NL, Berger RL, Tajima KA. Differential heat conduction microcalorimeter for heat-capacity measurements of fluids. *J Biochem Biophys Methods* 1993;26:149-71.

Gershfeld NL, Mudd CP, Tajima K, Berger RL. Critical temperature for unilamellar vesicle formation in dimyristoyl-phosphatidylcholine dispersions from specific heat measurements. *Biophysical Journal* 1993 (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10359-03 BEI
PERIOD COVERED October 1, 1992 to September 30, 1993		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Flash Photolysis Apparatus		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Allen Markowitz, M.S. Electrical Engineer ACES, BEIP, NCRR Burton Chidakel, B.S. Instrument Maker BEIP, NCRR		
COOPERATING UNITS (if any) LMC, DCE, NCI (F. Friedman, R. Robinson, Y. Omata, A. Koley)		
LAB/BRANCH Biomedical Engineering and Instrumentation Program		
SECTION Applied Clinical Engineering Section, CCIS		
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, Maryland 20892		
TOTAL STAFF YEARS: 0.5	PROFESSIONAL: 0.3	OTHER: 0.2
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews ☐ (b) Human tissues ☒ (c) Neither		
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.) This instrument is used to measure the kinetics of carbon monoxide (CO) binding to cytochrome P450 in liver microsomes from rats treated with various drugs and carcinogens. When CO is added directly to rat liver microsomes, the absorbance change at 450 nm occurs too rapidly to follow on a standard spectrophotometer. In order to observe this rapid reaction, a continuous dye-laser flash photolysis apparatus was constructed to monitor the kinetics of the absorbance change. Recombinant data generally were fit to a three-exponential kinetic model. However, each exponent included the contribution of several P450 forms. In order to determine the kinetic parameter for individual P450s, a difference kinetic method was used that employs the difference between the kinetic profiles in the absence and presence of a specific P450 effector. This new approach successfully yielded kinetic parameters for microsomal P450.		

OBJECTIVES: Recombination kinetics of CO following flash photolysis of the cytochrome P450-CO complex in rat liver microsomes was used to probe P450 structure and dynamics. The influence of various specific effectors of P450 function, such as anti-P450 antibodies and substrates, was used to elucidate the kinetic behavior of individual P450s. The general problem of attributing a rate constant to a specific P450 in the presence of multiple microsomal P450s was overcome by employing a difference kinetic method. This analysis is based on a kinetic profile which is the difference between the kinetic curves obtained in the presence and absence of the P450 effector.

The dye-laser flash photolysis apparatus was designed to measure the kinetic reactions.

METHODS EMPLOYED: The sample is pulsed with a laser flash at 530 nm to disrupt the photolabile bond between CO and the heme iron of the P450. Transmitted light at 450 nm is detected by the photomultiplier. Several optical filters and lenses select wavelength and focus the light. The photomultiplier voltage output is amplified, filtered, input into an analog-to-digital converter, and stored on the IBM AT. The amplifier with offset was designed to limit A/D quantification noise. The hardware filter was used to limit aliasing effects.

Data from repetitive experiments were averaged to improve the signal-to-noise ratio (S/N). Further S/N enhancement was obtained by a moving point average and fast Fourier transform techniques.

The CO recombinations were carried out under pseudo-first order conditions in excess (greater than tenfold) CO. Experimental data were processed and analyzed with the RS/1 software using least squares fitting to the multiexponential model.

MAJOR FINDINGS: The binding of CO to P450, with its characteristic absorbance change at 450 nm, was used as a probe for active site conformation. Such experiments have provided detailed information on the protein structure and dynamics in the heme region for several heme proteins, but they have been less extensively applied to the P450 system.

Microsomes contain a finite yet unknown number of P450s whose individual concentrations are also unknown. The capability to resolve these P450s is primarily determined by the sensitivity and noise of the instrumentation, which will influence the least squares analysis and thus the minimum number of exponentials to fit the data.

To overcome this problem, we circumvented the major problem of attempting to fit a minimum number of exponentials to a larger number of P450s. Instead, we employed a procedure that evaluates the difference between kinetic curves in the presence and absence of effector, thus cancelling the contributions from the majority of reactive P450s that do not interact with effector.

SIGNIFICANCE: In conjunction with separately derived equilibrium binding data, the kinetic data will help to provide a more comprehensive view of the P450-substrate-CO system. From the perspective of P450 dynamics, the kinetics of CO recombination after flash photolysis is a powerful probe for the accessibility of small molecules to the heme in the protein interior. The parameters obtained by this approach include the rates at which CO diffuses from bulk solvent into the protein interior. They also yield the relative amplitudes of kinetically distinguishable pathways toward the final P450-CO complex. These parameters reflect both the conformational structure and the dynamics of the P450.

The contribution of a given P450 to the parameter of interest (e.g., enzymatic activity) can be gauged in the presence of a microsomal mixture of P450s by simply performing measurements in the absence and presence of specific P450 effectors. The results demonstrate that individual P450s can be characterized in their native membrane environment using differential analytical procedures.

PROPOSED COURSE: Data testing is presently being performed on rat liver microsomes.

PUBLICATIONS: Markowitz A, Robinson RC, Omata Y, Friedman FK. A flash photolysis instrument with digital smoothing of data using a fast Fourier transform. Analytic Instrumentation 1992;20(4):213-21.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10360-03 BEI
PERIOD COVERED October 1, 1992 to September 30, 1993		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Motility of Tumor Cell Metastases		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation) Richard Chadwick, Ph.D. Head, Theor. Biomech. Group OD, BEIP, NCRR		
COOPERATING UNITS (if any) LP, NCI (L. Liotta, S. Aznavoorian); Pennsylvania State University (C. Dong)		
LAB/BRANCH Biomedical Engineering and Instrumentation Program		
SECTION Office of the Director		
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, Maryland 20892		
TOTAL STAFF YEARS: 0.2	PROFESSIONAL: 0.2	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews ☒ (b) Human tissues ☐ (c) Neither		
SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.) Invasion and metastatic spread of malignant neoplasms are associated with active locomotion of tumor cells. This locomotion occurs at the site of the primary growth, as well as at the entrance to and the egress from blood vessels. Some factors derived from host tissues have been shown to stimulate the intrinsic motility of tumor cells in <i>in vitro</i> studies. These factors are believed to influence both the extent and the direction of tumor cell movement <i>in vivo</i> to specific target organs. Although much experimental research has been done to observe this phenomenon, the detailed molecular kinetics and biomechanical functions of the generation of cell locomotion have not been well understood (e.g., initiation and regulation of pseudopod formation under certain chemoattractants). To gain such an understanding, we are using micropipette assays to characterize individual cell behavior, from which a dynamic process of pseudopod growth can be monitored under the light microscope. The information obtained can be used for developing mathematical models through a biophysical approach, based on both molecular and continuum mechanics theories, in order to understand the mechanism of this metastatic spread.		

OBJECTIVES: To use combined experimental and theoretical work to establish more definitely the biophysical processes of chemotactic responses of tumor cells.

SIGNIFICANCE: The ability of cells to perform active movements is vital to their functions in physiological and pathological processes. In tumor metastases, the cell projects pseudopods in a chemotactic gradient and migrates across tissues. Both this chemoattractant-stimulated motility and the formation of pseudopodia occur on a wide variety of adhesive substrata, suggesting that certain intrinsic motility events are independent of the attachment mechanism. The questions are then focused on our understanding of this active cell motility.

MAJOR FINDINGS: The human melanoma cell line, A2058, was used for our investigation. Chemotactic response of A2058 cells to type IV collagen (one of the extracellular matrix components) shows that the motility of the tumor cell is the principal requirement for metastasis. The first step of tumor spread occurs when a tumor cell forms a pseudopod toward a chemotactic source. Using micropipette technique, we filled a 5- to 6-mm-diameter pipette with type IV collagen and placed it near a cell. We have observed that under controlled (i.e., drug treatment-free) conditions, the pseudopod grows continuously into the pipette. The time course of the pseudopod growth is about two hours. If the cell is pretreated with pertussis toxin (PT), pseudopod growth is decreased significantly, suggesting that a signal transduction through the PT-sensitive G-protein pathway is involved. Blocking this signal could be important to the inhibition of tumor cell motility, which leads to cancer metastasis.

PROPOSED COURSE: To develop a new *in vitro* assay using micropipette technique to observe a dynamic growth of the pseudopod when a tumor cell is activated. Developing a model of the protrusion of pseudopodia in tumor cell metastasis is the next step, based on an assumption that actin filaments elongate due to actin polymerization at the tip of the pseudopod. The driving force of extension is hypothesized as being provided by the actin polymerization and osmotic swelling. The theoretical prediction of the time course of pseudopod growth will be validated by our experimental observation from the micropipette test.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10361-03 BEI
PERIOD COVERED October 1, 1992 to September 30, 1993		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Rheology of Sickle Erythrocytes		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Richard Chadwick, Ph.D. Head, Theor. Biomech. Group OD, BEIP, NCRR		
COOPERATING UNITS (if any) LCB, NIDDK (A. Schechter); Pennsylvania State University (C. Dong)		
LAB/BRANCH Biomedical Engineering and Instrumentation Program		
SECTION Office of the Director		
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, Maryland 20892		
TOTAL STAFF YEARS: 0.2	PROFESSIONAL: 0.2	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews ☒ (b) Human tissues ☐ (c) Neither		
SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.) The rheological properties of normal erythrocytes appear to be determined largely by those of the red cell membrane. In sickle cell disease, the intracellular polymerization of sickle hemoglobin upon deoxygenation leads to a marked increase in intracellular viscosity and elastic stiffness, and also has indirect effects on the cell membrane. In order to estimate the components of abnormal cell rheology due to the polymerization process (and that due to the membrane abnormalities), we have developed a mathematical model of whole cell deformability in narrow vessels. This model uses hydrodynamic lubrication theory to describe the pulsatile flow in the gap between a cell and the vessel wall. We use published values of normal and sickle cell membrane elastic modulus and of sickle hemoglobin viscous and elastic moduli as a function of oxygen saturation to estimate the cell deformation and relative hydrodynamic resistance as a function of oxygen saturation for sickle erythrocytes. The model should be useful for reconciling the vast and disparate sets of data available on the abnormal properties of sickle cell hemoglobin and sickle erythrocyte membranes--the two main factors that lead to pathology in patients with this disease.		

OBJECTIVES: To increase our understanding of the relative contributions of the red cell membrane and the internal sickle hemoglobin solution to the viscous and elastic components of total cell deformability during passage through the microcirculation, particularly at different oxygen saturation levels.

SIGNIFICANCE: The deformability of erythrocytes is a critical determinant of flow in the microcirculation. Abnormalities of these properties are thought to be the principal cause of the pathophysiology of sickle cell disease and other hemoglobinopathies. Extensive clinical studies of the effects of the abnormal sickle erythrocytes in patients have been reported. A central challenge in sickle cell rheology remains, however: to ascertain the relative importance of the sickle hemoglobin and cell membrane to the overall properties of sickle cells in the microcirculation.

MAJOR FINDINGS: The model indicates that a cell becomes less and less deformable when oxygen saturation drops below 40% to 50%, or when polymer fraction rises above 0.4, if the membrane rigidity has a value representative of a normal cell. The flow resistance of the cells increases significantly when oxygen saturation decreases to this critical level. Cell membrane elasticity certainly becomes a major determinant of the overall cell deformability when sickled hemoglobin solution is fully oxygenated. Several factors can contribute to altering the critical value of polymer fraction (or oxygen saturation). For example, increasing the oscillatory frequency of the blood flow tends to shift the critical value of polymer fraction downward. A membrane rigidity dependence on oxygen saturation can significantly alter this value, as well; in fact, the concept of a critical polymer fraction no longer applies. In other words, small amounts of polymer can significantly affect whole cell deformability in this case.

PROPOSED COURSE: The model will be extended to take into account the situation in which a cell passes through a very narrow capillary (i.e., the tube dimension is smaller than that of the undeformed particle). In such a case, the bending rigidity of the cell membrane may play an important role. A filtration assay of sickle cell trait suspensions using nickel mesh will be designed to investigate filtration under oscillatory conditions.

PUBLICATIONS: Dong C, Chadwick RS, Schechter AN. Influence of sickle hemoglobin polymerization and membrane properties on deformability of sickle erythrocytes in the microcirculation. Biophysical Journal 1992;63:774-83.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10362-03 BEI
PERIOD COVERED October 1, 1992 to September 30, 1993		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Binding Forces in Receptor-Mediated Cell Adhesion		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation) Alec Eidsath, Ph.D. Staff Fellow MES, BEIP, NCRR		
COOPERATING UNITS (if any) LTCB, DCE, NCI (C. Saxinger)		
LAB/BRANCH Biomedical Engineering and Instrumentation Program		
SECTION Mechanical Engineering Section		
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, Maryland 20892		
TOTAL STAFF YEARS: 0.5	PROFESSIONAL: 0.5	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews ☒ (b) Human tissues ☐ (c) Neither		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Since it is known that the tat protein of the HIV will enter the cell in vitro and stimulate the production of HIV products, there is almost certainly a receptor site for it on the cell membrane. Segments of this 86 amino acid protein are being synthesized to determine which segment is responsible for binding to the receptor. This phenomenon will be evaluated by quantitatively measuring the binding force between the various segments and several types of human cells grown in tissue culture. We hope to use a flow cell and image analysis to determine the average shear force needed to detach the cells. The cells will then be attached to the bottom of a 96-well plate, which will be moved automatically from well to well under computer control.		

OBJECTIVES: To rank the binding energies of various tat fragments by determining the force necessary to remove bound cells.

METHODS EMPLOYED: We have chosen two methods of applying force to a cell: centrifugal force and shear force. For the experiments using centrifugal force, we will bind cells to polypeptides bound to the bottoms of 96-well plates, and then spin the cells in a medium of a higher specific gravity. Cells that are pulled off are then aspirated, and the number of bound cells is measured using a fluorescence reader. This method is limited by the force that can be put onto the microtitre plates themselves. Fragments that are more tightly bound can then be removed by centrifugal force, and will be analyzed using a flow system, which can generate strong shear forces. Using the flow system together with a microscope makes possible the direct viewing of how the cells deform and eventually separate from the wall. Since the synthesis of the fragments takes place in a 96-well plate, it is very convenient to base the design of the flow cell on the geometry of the plate. We have decided to use a stagnation point flow (a jet of liquid impinging on the surface containing the cells). The shear stress is inversely proportional to the radius, so one would expect to see a clear area near the center of the well where the flow was stronger than the binding energy, and then a resumption of attached cells at a radius where the binding force just overcomes the shear force. A commercial image-analysis system is used to automatically determine this critical radius (the cells will be loaded with a fluorescent dye, and the image will be taken in an epifluorescence setup). The instrument has been designed to fit into a flow hood inside a biohazard room, due to the potential dangers of the virus being studied.

SIGNIFICANCE: We think that the binding studies are an excellent tool to complement fundamental molecular biology studies. The direct aim of the project is to determine which amino acids are involved in the binding of the cell to the surface receptor. We intend to compare these results to the three-dimensional configurations produced using molecular dynamics programs. This research will also enable us to better understand the basic immunology of the virus.

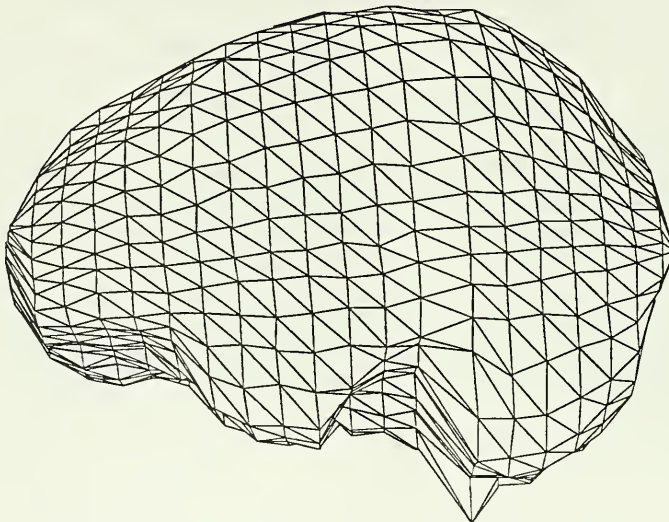
PROPOSED COURSE: We are in the process of completing the final experiments using the centrifugal force field. The stagnation point flow device has been completed, and a preliminary test using a single well was satisfactory. By the end of the summer, the instrument should be complete.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10363-03 BEI
PERIOD COVERED October 1, 1992 to September 30, 1993		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Current Dipole Localization Using EEG Data Model		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation) Bradley Roth, Ph.D. Senior Staff Fellow MES, BEIP, NCRR David Kovar, B.S. Summer Student MES, BEIP, NCRR		
COOPERATING UNITS (If any) MNE, NINDS (M. Hallett, S. Sato, L. Cohen, A. Gorbach); Department of Neurology, Georgetown University Hospital (M. Balish)		
LAB/BRANCH Biomedical Engineering and Instrumentation Program		
SECTION Mechanical Engineering Section		
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, MD 20892		
TOTAL STAFF YEARS: 0.3	PROFESSIONAL: 0.3	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) _ (a) Human _ (b) Human X (c) Neither subjects tissues _ (a1) Minors _ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) A computer program will be developed that can determine the position, orientation, and strength of a current dipole in the brain from electroencephalographic or magnetoencephalographic data. The electrical properties of the head will be modeled using a three-layer model: brain, skull, and scalp. A realistic head shape will be used. The program will be used to localize the focus of epileptic discharges and to analyze evoked potentials. Comparisons will be made between the realistically shaped head model and the three-sphere model.		

OBJECTIVES: To localize an equivalent dipole source in the brain, using electroencephalographic (EEG) or magnetoencephalographic (MEG) data.

SIGNIFICANCE: The localization of the source of the EEG and MEG is an old problem that has been studied extensively. Until recently, the primary model used to analyze EEG and MEG data was the three-sphere model. It has become clear recently that there are significant errors introduced by modeling the head as a sphere. By using a realistic head geometry, more accurate localizations can be obtained, thus improving our understanding of human cortical organization and enabling surgeons to have more precise knowledge of the areas of the brain responsible for epileptic seizures.

MAJOR FINDINGS: Comparisons between the three-sphere model and the realistically shaped head model indicate that average differences of 2 cm are observed between the two models in the predicted dipole position.



The distribution of 1600 triangles over the brain surface, used to calculate the electrical potential produced by an electric dipole with the realistically shaped head model. The coordinates of the triangles' vertices were obtained by MRI of the subject's head.

PROPOSED COURSE: The first goal will be to verify the model by comparing it to the three-sphere model. Then the sensitivity of the model to various parameters, such as skull conductivity, will be determined. Random noise will be added to the simulations to

determine the influence of measurement noise on the results. Finally, the model will be used to analyze experimental data.

PUBLICATIONS: Roth BJ, Sato S. Accurate and efficient formulas for averaging the magnetic field over a circular coil. In: Hoke M, Erne SN, Okada TC, Romani GL, eds. Biomagnetism: clinical aspects. Amsterdam: Elsevier, 1992;797-800.

Roth BJ, Balish M, Gorbach A, Sato S. How well does the three-sphere model predict dipoles in a realistically shaped head? Electroenceph Clin Neurophysiol (in press).

Toro C, Matsumoto J, Deuschl G, Roth BJ, Hallett M. Source analysis of scalp-recorded movement-related electrical potentials. Electroenceph Clin Neurophysiol 1993;86:167-75.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10365-03 BEI
PERIOD COVERED October 1, 1992 to September 30, 1993		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Study of Polarized Light Propagation in Scattering Media		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Joseph M. Schmitt, Ph.D. Staff Fellow ACES, BEIP, NCRR Robert F. Bonner, Ph.D. Biophysicist EEES, BEIP, NCRR		
COOPERATING UNITS (if any) PSL, DCRT (A. Gandjbakhche)		
LAB/BRANCH Biomedical Engineering and Instrumentation Program		
SECTION Applied Clinical Engineering Section		
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, Maryland 20892		
TOTAL STAFF YEARS: 0.2	PROFESSIONAL: 0.2	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews ☐ (b) Human tissues ☒ (c) Neither		
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.) Building on results of our earlier theoretical work elucidating the conditions under which photons maintain their polarization after being scattered multiple times, we have been developing increasingly sensitive methods for measuring small polarization changes of light propagating in a multiple scattering media. A novel reflection polarimeter, developed in our lab, enables the measurement of physiological concentrations of glucose in aqueous solution over pathlengths of only a few millimeters, using a solid-state reflection-mode sensor.		

OBJECTIVES: To address fundamental questions concerning the propagation of polarized light in scattering media, and to investigate new techniques for quantifying regional concentrations of optically active substances in tissues.

METHODS EMPLOYED: Our basic experimental apparatus consists of an ultrasonic polarization modulation and detection system, capable of measuring extremely small polarized light intensities in a background of scattered, depolarized light. To simulate light scattering in biological tissues, samples containing polystyrene beads suspended in water are used. The experimental results are compared with results obtained from Monte Carlo simulations, which quantify the effects of the size and concentration of the scattering particles on the polarization transformations that the light undergoes as it propagates through a sample.

We recently introduced a new type of polarimetry that allows small optical rotations to be measured with a simpler and less expensive apparatus. Light is reflected from the glass walls of the cuvette containing the sample at an angle slightly greater than Brewster's angle, and is detected by a pair of differentially connected photodiodes. The recorded signal is linearly proportional to the optical rotation multiplied by a gain factor that is a function of the reflection angle.

MAJOR FINDINGS: Our experiments demonstrate that light propagating in a dense medium with optical properties similar to those of biological tissues loses over 99% of its initial polarization over a distance of less than one millimeter. In spite of this rapid polarization loss, we found that measurements of the polarization state of light could be made through blood and skin-tissue phantoms as thick as 5 mm. We found that, in accordance with theoretical predictions, circularly polarized light propagates farther than linearly polarized light in tissue-like media, and that decay of polarization versus optical thickness has a strong dependence on particle size.

SIGNIFICANCE: Little is known about the propagation of polarized light in optically dense substances. This study has provided new insights into the effects of multiple scattering on polarized-light propagation. Our most recent findings suggest that polarization measurements may be useful for measuring the sizes of cells in a dense suspension and for characterizing the birefringence of tissues *in situ*. The reflection polarimeter used in our basic studies may have a broader range of applications in analytical chemistry.

PROPOSED COURSE: Several important questions remain regarding the differential behavior of the propagation of circularly and linearly polarized light. To help answer these questions, we intend to incorporate vector polarization transformations into our present (scalar) Monte Carlo simulation program. A new set of experiments using improved instrumentation is planned to verify

the simulation results. At the same time, we plan to investigate cell-sizing applications of the polarization measurements.

PUBLICATIONS: Schmitt JM, Gandjbakhche AH, Bonner RF. Use of polarized light to discriminate short-path photons in a multiply scattering medium. Applied Optics 1992;31:6535-46.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10367-03 BEI
PERIOD COVERED October 1, 1992 to September 30, 1993		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Imaging of Biological Tissues Using High-Frequency, Intensity-Modulated Light		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Joseph M. Schmitt, Ph.D. Staff Fellow ACES, BEIP, NCRR		
COOPERATING UNITS (if any) IR CE, NHLBI (A. Knüttel, R. Balaban); IR LCB, NHLBI (J. Knutson)		
LAB/BRANCH Biomedical Engineering and Instrumentation Program		
SECTION Applied Clinical Engineering Section		
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, Maryland 20892		
TOTAL STAFF YEARS: 0.5	PROFESSIONAL: 0.5	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews ☐ (b) Human tissues ☒ (c) Neither		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Our efforts this year were concentrated on identifying the practical limitations of diffusing-wave interference spectrophotometry in the context of thick-tissue imaging for clinical application. This goal was achieved by using a combination of theoretical and experimental investigations. We found that although it is possible to determine very precisely the position of a single object embedded deep in a homogeneous medium, the resolution of two or more absorbing (or fluorescent) objects is limited by the width of the diffusive-wave point-spread function, which exceeds about 5 mm at the highest modulation frequencies studied.		

OBJECTIVES: To investigate methods of imaging the concentration of intrinsic absorbers or contrast agents imbedded in living tissue using diffusive light waves. In particular, to investigate the application and limitations of techniques based on interfering high-frequency (100 MHz to several GHz) modulated waves.

METHODS EMPLOYED: Several improvements were incorporated into the experimental setup to facilitate investigations of practical imaging configurations. First, a computer-controlled gated CCD camera was designed and built to make simultaneous measurements of the phase and magnitude of modulated light re-emitted from a tissue phantom. Next, acousto-optic modulators were configured to allow two input beams of variable phase and magnitude to be scanned electronically across the phantom. Using this setup, we performed experiments under conditions similar to those under which measurements would need to be made from human skin in the clinic.

To investigate the limitations of the contrast and resolution achievable using the setup, measurements were made on liquid phantoms containing two embedded absorbers. A small number of studies were also carried out using chicken-breast meat containing embedded capsules of hemoglobin. We performed a series of numerical and analytical simulations to aid interpretation of the measured data and to study the effect of variables that were not conducive to experimental study. A novel analytical model, based on an embedded periodic grating, was employed to quantify contrast and resolution.

MAJOR FINDINGS: The results of an extensive series of experimental and theoretical studies have enabled us to define the capabilities and limitations of diffusive-wave imaging. We found that by using an optical balancing technique, the position of a single object embedded more than a centimeter deep in a homogeneous tissue phantom could be determined very precisely. However, resolution of two or more absorbing (or fluorescent) objects was found to be limited by the width of the diffusive-wave point-spread function, which exceeds about 5 mm at the highest modulation frequencies studied. The ability to resolve embedded objects was also found to be limited by the scattering inhomogeneities located close to the surface when using a small-aperture detector. It appears that the diffusive-wave interference techniques developed to date are most suitable for localized spectroscopy or characterization of tissues on a spatial scale on the order of the depth of the sample volume of interest.

SIGNIFICANCE: In the last few years, many university and research laboratories have initiated efforts directed at developing optical-based tissue imaging and spectroscopy methods. Our approach in this study has been to confront two of the major problems encountered when light is used to probe tissue: poor sensitivity to deep structures, and nonlocalizability of the sample volume. Our results have shown that these problems can be alleviated to a certain extent by taking advantage of the

properties of interfering high-frequency-modulated diffusive waves. Unfortunately, because of the long wavelength of diffusive waves in a tissue, the volume of the region over which the waves interfere at points deep in a tissue cannot be tightly confined. As a result, the minimum distance between resolvable objects is larger than that desired in most applications of clinical importance. The conclusions of this study are quite general and have implications for all imaging and spectroscopy methods that rely on the measurement of diffuse light intensity.

PROPOSED COURSE: We plan to explore the application of high-frequency, intensity-modulated light in localized spectroscopy, rather than in imaging *per se*, as we originally proposed. This exploration will require the development of multiple-wavelength light sources and detection systems for extracting spectra of hemoglobin and other intrinsic chromophores in tissue. We intend to test several methods for obtaining derivative spectra, which combine modulation of the wavelength and amplitude of light.

PUBLICATIONS: Schmitt JM, Knüttel A, Knutson JR. Interference of diffusive light waves. *J Opt Soc Am* 1992;9:1832-43.

Knüttel A, Schmitt JM, Knutson JF. Spatial localization of absorbing bodies by interfering diffusive photon-density waves. *Applied Optics* 1993;32:381-9.

Knüttel A, Schmitt JM, Knutson JF. Acousto-optic scanning and interfering photon density waves for the precise localization of an absorbing (or fluorescent) body in a turbid medium. *Rev Sci Instrum* 1993;64:638-44.

Knüttel A, Schmitt JM, Knutson JF. Improvement of spatial resolution in reflectance near-IR imaging by laser-beam interference. In: *Proceedings of the SPIE*. 1992;1640:405-16.

Knüttel A, Schmitt JM, Barnes R. Spatial localization using photon-density waves: contrast enhancements and limitations. In: *Proceedings of the SPIE*. 1993 (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10372-02 BEI
PERIOD COVERED October 1, 1992 to September 30, 1993		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Development of a Chaos PC Resource at the NIH		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) James R. Ellis, Ph.D. Electronics Engineer ACES, BEIP, NCRR Jim Gruber, A.A. Electronics Technician BEIP, NCRR		
COOPERATING UNITS (if any) LB, NCRR (E. Cerutti)		
LAB/BRANCH Biomedical Engineering and Instrumentation Program		
SECTION Applied Clinical Engineering Section		
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, MD 20892		
TOTAL STAFF YEARS: 0.1	PROFESSIONAL: 0.1	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) _ (a) Human subjects _ (a1) Minors _ (a2) Interviews _ (b) Human tissues X (c) Neither		
SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.) Biological organisms are nonlinear, time-varying systems with many variables. It is generally difficult to obtain clean, simple input-output relationships or low-variance correlation relationships. There is a growing community of workers who believe that the developing field of dynamical systems, especially its best-known areas of chaos and fractals, offers attractive alternative methods to the use of linear systems with additive noise for modeling and studying biological and biomedical relationships. By making low-cost, computer-based materials available to the entire NIH community, we hope to further two goals. One is to allow interested researchers to obtain a fuller understanding of the principles and results available. The other is to facilitate limited trials of particular combinations of processing algorithms, parameters, and data sets to determine if chaos, fractals, or other aspects of dynamical systems might be worthwhile in a given problem area. The NIH Library, a unit of NCRR, agreed to develop a campus-wide resource for chaos and dynamical systems programs for the personal computer world, in cooperation with the Inter-Institute Chaos Council of the NIH.		

OBJECTIVES: To develop an NIH campus-wide resource for demonstrating and sharing chaos and dynamical systems programs for the personal computer world.

METHODS EMPLOYED: The NIH Library and the Chaos Club at NIH held preliminary discussions concerning an NIH-wide resource of literature and computer resources. The BEIP assumed responsibility for advising the NIH Library on acquisition of hardware and software for a campus-wide Chaos Personal Computer Program Resource, in cooperation with the Chaos Club (now the Inter-Institute Chaos Council, or IICC) at the NIH. The BEIP prepared a list of desired PC software and hardware in consultation with the IICC. Library personnel upgraded the hardware and software, and the BEIP aided in checking out the system and making it available to the NIH community through the IICC.

SIGNIFICANCE: Dynamical systems are likely to be extremely important in biology in the near to midterm future. By making low-cost, computer-based materials available to the entire NIH community, we hope to further two goals. One is to allow interested researchers to obtain a fuller understanding of the principles and results available. The other is to facilitate limited trials of particular combinations of processing algorithms, parameters, and data sets to determine if chaos, fractals, or other aspects of dynamical systems might be worthwhile in a given problem area. This resource can help to make the mathematics, physics, and computer science associated with chaos and dynamical systems more accessible to biologists, chemists, and medical doctors.

PROPOSED COURSE: The NIH Library has taken over support of this resource for the biomedical user community, as projected. The BEIP's responsibilities to aid in acquisition and installation have been completed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10373-03 BEI																					
PERIOD COVERED October 1, 1992 to September 30, 1993																							
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Operation of the <i>In Vivo</i> NMR Research Center																							
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">Chrit Moonen, Ph.D.</td> <td style="width: 33%;">Manager</td> <td style="width: 33%;">NMR, BEIP, NCRR</td> </tr> <tr> <td>Guoying Liu, Ph.D.</td> <td>Postdoctoral Fellow</td> <td>NMR, BEIP, NCRR</td> </tr> <tr> <td>Geoffrey Sobering, Ph.D.</td> <td>Senior Staff Fellow</td> <td>NMR, BEIP, NCRR</td> </tr> <tr> <td>Alan Olson, M.S.</td> <td>Electronics Engineer</td> <td>NMR, BEIP, NCRR</td> </tr> <tr> <td>Daryl DesPres, B.S.</td> <td>Biologist</td> <td>NMR, BEIP, NCRR</td> </tr> <tr> <td>Denise Sobering, B.A.</td> <td>Secretary</td> <td>NMR, BEIP, NCRR</td> </tr> <tr> <td>Yoseph Shiferaw, Ph.D.</td> <td>Staff Fellow</td> <td>NMR, BEIP, NCRR</td> </tr> </table>			Chrit Moonen, Ph.D.	Manager	NMR, BEIP, NCRR	Guoying Liu, Ph.D.	Postdoctoral Fellow	NMR, BEIP, NCRR	Geoffrey Sobering, Ph.D.	Senior Staff Fellow	NMR, BEIP, NCRR	Alan Olson, M.S.	Electronics Engineer	NMR, BEIP, NCRR	Daryl DesPres, B.S.	Biologist	NMR, BEIP, NCRR	Denise Sobering, B.A.	Secretary	NMR, BEIP, NCRR	Yoseph Shiferaw, Ph.D.	Staff Fellow	NMR, BEIP, NCRR
Chrit Moonen, Ph.D.	Manager	NMR, BEIP, NCRR																					
Guoying Liu, Ph.D.	Postdoctoral Fellow	NMR, BEIP, NCRR																					
Geoffrey Sobering, Ph.D.	Senior Staff Fellow	NMR, BEIP, NCRR																					
Alan Olson, M.S.	Electronics Engineer	NMR, BEIP, NCRR																					
Daryl DesPres, B.S.	Biologist	NMR, BEIP, NCRR																					
Denise Sobering, B.A.	Secretary	NMR, BEIP, NCRR																					
Yoseph Shiferaw, Ph.D.	Staff Fellow	NMR, BEIP, NCRR																					
COOPERATING UNITS (if any) None																							
LAB/BRANCH Biomedical Engineering and Instrumentation Program																							
SECTION <i>In Vivo</i> NMR Research Center																							
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, MD 20892																							
TOTAL STAFF YEARS: 2.7	PROFESSIONAL: 1.3	OTHER: 1.4																					
CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">X ☐ (a) Human subjects</td> <td style="width: 33%;">☐ (b) Human tissues</td> <td style="width: 33%;">☐ (c) Neither</td> </tr> <tr> <td>☐ (a1) Minors</td> <td></td> <td></td> </tr> <tr> <td>☐ (a2) Interviews</td> <td></td> <td></td> </tr> </table>			X ☐ (a) Human subjects	☐ (b) Human tissues	☐ (c) Neither	☐ (a1) Minors			☐ (a2) Interviews														
X ☐ (a) Human subjects	☐ (b) Human tissues	☐ (c) Neither																					
☐ (a1) Minors																							
☐ (a2) Interviews																							
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The purposes of the <i>In Vivo</i> NMR Research Center are the advancement of <i>in vivo</i> NMR technology; its applications to animal and human physiology; and, ultimately, its application in the clinical setting. The NMR Center's staff collaborate with NIH and other investigators on new methods and original application of functional magnetic resonance methods, including flow imaging; imaging of capillary circulation; diffusion of water and metabolites in brain and muscle; imaging of oxygen consumption; imaging and spectroscopy of aerobic and anaerobic glucose metabolism and transport; imaging of altered metabolism in the brain, such as the types of anomalies due to tumors, Alzheimer's disease, and multiple sclerosis; and localized spectroscopy to follow metabolism and pharmacokinetics. Day-to-day operations of the <i>In Vivo</i> NMR Research Center include (but are not limited to) scheduling of usage of magnets and computers, ordering supplies stocked by the Center for all users, and other activities necessary for a shared resource. In addition, various tasks are directed at the basic operation of any laboratory, such as accounting for usage and funding, submission of necessary documentation, telephone inquiries, and information dissemination.																							

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER 201 RR 10378-02 BEI
PERIOD COVERED October 1, 1992 to September 30, 1993		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Mechanisms of Angioplasty and Atherectomy of Coronary Stenoses		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Robert Bonner, Ph.D. Physicist EEES, BEIP, NCRR		
COOPERATING UNITS (if any) CB, NHLBI (S. Epstein); Washington Cardiology Center; SCIMED, Inc.; CVIS, Inc.		
LAB/BRANCH Biomedical Engineering and Instrumentation Program		
SECTION Electrical and Electronic Engineering Section		
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, MD 20892		
TOTAL STAFF YEARS: 0.3	PROFESSIONAL: 0.3	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews ☐ (b) Human tissues ☒ (c) Neither		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) In an effort to develop new, more effective forms of angioplasty, we have sought to better understand the nature of focal coronary stenoses and their acute and chronic responses to intervention. Through the development of the clinical use of intravascular ultrasound (IVUS), we have shown that the composition and stiffness at focal chronic stenoses is remarkably different from adjacent angiographically normal vessel segments, whereas the total mass of atheroma is remarkably similar. The principal lesions currently treated by angioplasty have become rigid due to fibrosis and calcification, which prevent compensatory dilatation observed in more compliant (though heavily diseased) segments. Accordingly, successful interventions disrupt/alter the stiff constraining elements to irreversibly make them sufficiently compliant to permit vessel expansion. Successful therapy requires segmental (limited) rupture of the stiff annulus and creation of a large compliant arc. Acoustic transients created by rapid bubble expansion are the principal cause of this disruption in laser angioplasty. Directional atherectomy creates deep focal excisions that can make a small arc (~60°) highly compliant. Rotabators remove luminal calcification, thereby reducing wall stiffness. Following the expansion of "rigid" metallic stents at high pressures, the surrounding tissues generate significant compressive forces, causing an acute and chronic compressive narrowing of treated lesions that may be the major cause of restenosis. Transient, moderate (~60°C) thermal elevations associated with thermal angioplasty elicit a profound dose-dependent proliferative response similar to that seen with severe mechanical injury.		

OBJECTIVES: Current objectives are: (1) to determine the mechanisms of success and failure of angioplasty/atherectomy with devices now being used clinically; (2) to design new devices and methods of angioplasty, based on this new understanding; (3) to develop guidelines for optimal selection of available devices, based on specific lesion characteristics; and (4) to decrease restenosis rate.

METHODS EMPLOYED: Our work was focused on quantitative morphometry based on both clinical intravascular ultrasound measurements and pathology of atherectomy specimens and postmortem coronaries, and the development of animal models of proliferative responses to vascular injury and *in vitro* assessment of device performance. Final evaluation of the angioplasty devices will be based on quantitative intravascular ultrasound examination of clinical lesions before and after therapy, in conjunction with long-term follow-up.

MAJOR FINDINGS: (1) We have shown that the composition and stiffness at focal chronic stenoses is remarkably different from adjacent, angiographically normal vessel segments, whereas the total mass of atheroma is remarkably similar. (2) The principal lesions currently treated by angioplasty have become stiff due to fibrosis and calcification, which prevent compensatory dilatation observed in more compliant (though heavily diseased) segments. (3) Successful intervention relies on the disruption/alteration of stiff constraining elements to make them sufficiently and irreversibly compliant, thus permitting acute and chronically maintained vessel expansion. Successful angioplasty, therefore, works by segmental (limited) rupture of the stiff annulus and the creation of a large compliant arc. (4) Acoustic transients created by rapid bubble expansion during pulsed-laser angioplasty similarly disrupt the stiff vessel segment. Since the volume directly disrupted by the large pressure transients is >100 times the volume vaporized, it is clear that pulsed laser angioplasty is a potent disrupting procedure. Thus, dissection ensues largely from efforts to increase the amount of tissue vaporization, thereby subjecting each plaque element to many (>30) pressure transients. More rapid advancement of the laser angioplasty catheter is proposed to minimize the depth of tissue disruption in order to minimize severe dissection. (5) Directional atherectomy can create a deep focal excision that makes a small arc (~60°) highly compliant, frequently to the point of becoming a pseudo-aneurism. (6) Rotablaters remove a luminal layer of calcification, subsequently allowing relatively low pressure disruption of the remaining lesion. (7) Following the expansion of "rigid" metallic stents at high pressures, the surrounding tissues generate significant compressive forces that cause significant acute and chronic recoil. (8) These findings suggest that acute and chronic compressive narrowing of treated lesions may be a major contributor to restenosis. (9) In animal models of restenosis, we have demonstrated that transient, moderate (~60°C) thermal elevations associated with thermal angioplasty elicit a

profound, dose-dependent proliferative response similar to that seen in severe mechanical injury.

SIGNIFICANCE: Development of more efficacious means of relieving atherosclerotic obstructions in human blood vessels (particularly in the coronary arteries) or reducing restenosis could have a dramatic effect on the practice of cardiology and vascular surgery. A greater understanding of the composition and structure of atherosclerotic stenoses (as determined by IVUS) and their responses to specific devices could lead to more efficacious selection of devices and refinement of technique. Dramatic improvements in lowering dissection rate have been observed in preliminary trials of new laser angioplasty methodology.

PROPOSED COURSE: Continued development of the IVUS database and analysis of pre- and postintervention vessel wall morphology in patients undergoing angioplasty. Quantitative testing of models of angioplasty and vessel wall compliance changes. Testing of drugs delivered locally to sites of vascular injury to inhibit smooth muscle cell proliferation in the animal models developed.

PUBLICATIONS: Douek PC, Correa R, Neville R, Unger EF, Shou M, Banai S, Ferrans VJ, Epstein SE, Leon MB, Bonner RF. Dose-dependent smooth muscle cell proliferation induced by thermal injury with pulsed infrared lasers. *Circulation* 1992;86:1249-56.

Leon MB, Bonner RF. Future directions in laser angioplasty. In: Topol E, ed. *Interventional cardiology*. 1993 (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 RR 10380-02 BEI

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pathophysiology of Syringomyelia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Alec Eidsath, Ph.D.	Staff Fellow	MES, BEIP, NCRR
Robert L. Dedrick, Ph.D.	Chief	CHES, BEIP, NCRR
Thomas Talbot, M.S.	Mechanical Engineer	ACES, BEIP, NCRR

COOPERATING UNITS (if any)

SNB, NINDS (E. Oldfield, J. Heiss)

LAB/BRANCH

Biomedical Engineering and Instrumentation Program

SECTION

Mechanical Engineering Section

INSTITUTE AND LOCATION

NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

0.2

PROFESSIONAL:

0.2

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

X ☐ (a) Human	☐ (b) Human	☐ (c) Neither
subjects	tissues	
☐ (a1) Minors		
☐ (a2) Interviews		

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Syringomyelia is a cavitation of the spinal cord. It most commonly occurs in association with structural abnormalities of the foramen magnum. We plan to assist SNB in the acquisition of pressures in the ventricle, syrinx, and spinal subarachnoid space. The data will be evaluated in connection with anatomic information obtained by cerebrospinal MRI, ultrasonography, and Imatron CT. Correlation of the anatomic and physiologic measurements should help to elucidate the mode of progression of syringomyelia, and may have implications for its treatment.

OBJECTIVES: This study seeks to determine the pathophysiology of the induction and progression of syringomyelia. Establishing the pathophysiology of this disease may have implications for its treatment.

METHODS EMPLOYED: A preoperative series of tests will be conducted in which pressures from the lumbar space and syrinx, along with the EKG trace, will be taken while the subject is awake. Pressures will be monitored during modified Queckenstedt tests (a cuff placed around the neck causing a transient increase in the intracerebral pressure), as well as during such common events as coughing and straining. During surgery, measurements will be obtained at three locations along the spine (the lumbar space, syrinx, and lateral ventricle) in addition to the main arterial and venous pressures. Data from the pressure transducers will be digitized by means of a general-purpose, analog-to-digital board controlled by a "virtual instrument" written in the LabView programming language. LabView was chosen because it gives an easy-to-use graphic interface to the data-collection procedure--a function considered essential to technicians in the operating room.

SIGNIFICANCE: The natural history of syringomyelia is one of gradual, progressive deterioration over many years. One objective of surgical treatment is to arrest the disease's progression.

PROPOSED COURSE: Eight patients have already been seen, and we are in the process of analyzing the data. One problem that must be addressed is how to automate the analysis of the large amount of data taken for each patient. Two additional patients are to be studied under this protocol. A presentation entitled "Cervical Subarachnoid Pressure Waves Promote Syringomyelia Progression" will be given at the Congress of Neurological Surgeons Annual Meeting, to be held in Vancouver, British Columbia, Oct 2-7, 1993.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10381-02 BEI
PERIOD COVERED October 1, 1992 to September 30, 1993		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Patient Electronic Monitoring System, Model II		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Horace E. Cascio, BSEE Electronics Engineer EEES, BEIP, NCRR George L. Hemphill, B.A. Engineering Technician EEES, BEIP, NCRR		
COOPERATING UNITS (if any) OD, CC (N. Kelly, R. Mack)		
LAB/BRANCH Biomedical Engineering and Instrumentation Program		
SECTION Electrical and Electronic Engineering Section		
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, Maryland 20892		
TOTAL STAFF YEARS: 0.70	PROFESSIONAL: 0.30	OTHER: 0.40
CHECK APPROPRIATE BOX(ES) X ☐ (a) Human ☐ (b) Human (c) Neither subjects tissues ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The Clinical Center has requested that the BEIP install two additional Patient Electronic Monitoring Systems (PEMS) on the 5E and 3E Nursing Units. This system, which alerts the nursing staff when a patient (or more than one) is leaving the nursing unit, is a welcome aid in the care of patients with Alzheimer's disease or other dementias. The system is based on a wristwatch-size radio transmitter worn by the patient. Detector units, placed at each of two exit doors, sense the presence of any patient wearing a transmitter watch. With the detection of a patient's transmitter, a microprocessor-based controller locks the exit door, activates an alarm, and sends the patient's name and location to a personal computer, which displays and records the time and date of each attempt a patient makes to leave the unit.		

OBJECTIVES: To redesign the original PEMS, currently operating on the 6D Nursing Unit, to include state-of-the-art technologies in order to increase system reliability and reduce maintenance.

METHODS EMPLOYED: An improved, printed-circuit patient identifier will be developed using surface-mount integrated circuits and chip resistors and capacitors. The circuit of the identifier will incorporate a crystal-controlled modulator circuit and will be powered by a rechargeable lithium battery. These features will practically eliminate the weekly maintenance routine. The control unit will use an embedded CPU card that allows software program development on a personal computer, in turn eliminating dependency on an obsolete Intel MDS Program Development System. All of the circuitry for the controller will be placed on printed circuit boards in a modular form.

SIGNIFICANCE: The redesign of the PEMS will increase system reliability and reduce the need for technical assistance.

PROPOSED COURSE: The installation of the 5E PEMS has been completed. The system has been released to the nursing staff for their use. The evaluation of the new Identifiers and of the system hardware will continue. The installation of the 3E PEMS is now in progress. Once the installation is completed, the system will be evaluated and the nurses will be instructed in its use.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10383-02 BEI
PERIOD COVERED October 1, 1992 to September 30, 1993		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Diffusion Coefficient Measurement		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) James Mattiello, Ph.D. Staff Fellow MES, BEIP, NCRR		
COOPERATING UNITS (if any) LDRR, OIR, OD (J. Frank)		
LAB/BRANCH Biomedical Engineering and Instrumentation Program		
SECTION Mechanical Engineering Section		
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, MD 20892		
TOTAL STAFF YEARS: 0.05	PROFESSIONAL: 0.05	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews ☐ (b) Human tissues ☒ (c) Neither		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Magnetic resonance imaging and spectroscopy are used to obtain a noninvasive measurement of the diffusion coefficient of water protons. This apparent diffusion coefficient of water samples is proportional to concentrations of gadolinium and dysprosium (MRI contrast enhancement agents). This investigation studies the magnitude and mechanism of this change in the apparent diffusion coefficient, and the ways this change could be used to measure the concentration of the parametric ions.		

MAJOR FINDINGS: For two different gadolinium contrast agents and one dysprosium contrast agent, the apparent diffusion coefficient changed as a function of the concentration. The gadolinium agents had different slopes, compared to the dysprosium curve. The magnitude of this slope would allow for the determination of the agent's concentration in the concentration range of 5 to 0.05 mMol.

This investigation of the apparent diffusion coefficient change for various concentrations of parametric and nonparametric agents has concluded. The change in diffusion coefficient was not sufficient to allow for accurate measurements of contrast concentration.

PROPOSED COURSE: This research study has concluded and further studies are not planned.

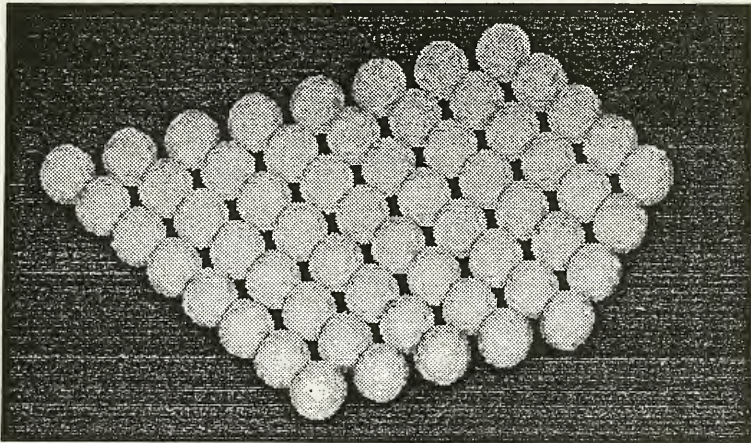
DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10384-02 BEI
PERIOD COVERED October 1, 1992 to September 30, 1993		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Contribution of Diffusion to Magnetization Transfer		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation) James Mattiello, Ph.D. Staff Fellow MES, BEIP, NCRR		
COOPERATING UNITS (if any) CE, NHLBI (R. Balaban); DR, CC (D. LeBihan)		
LAB/BRANCH Biomedical Engineering and Instrumentation Program		
SECTION Mechanical Engineering Section		
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, MD 20892		
TOTAL STAFF YEARS: 0.15	PROFESSIONAL: 0.15	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) _ (a) Human subjects _ (a1) Minors _ (a2) Interviews _ (b) Human tissues X (c) Neither		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Magnetization transfer contrast (MTC) is a technique that improves the contrast of magnetic resonance images by applying low-power radio-frequency irradiation, which is 10 kHz away from the main water proton resonance frequency, to excite macromolecular hydrogens. To determine if this rate of magnetization transfer is limited by diffusion of water protons, the temperature dependence of both diffusion and magnetization transfer were determined in the same material. Experiments were conducted on a 4.7-T GE Omega spectrometer; the temperature variation in the material was accomplished by circulating temperature-controlled water surrounding the sample. Spin-lattice relaxation times (T1) were determined with and without the RF saturation pulse. The rate of magnetization transfer for the material was then determined.		

MAJOR FINDINGS: T1 without the saturation pulse and diffusion coefficient have a linear dependence as a function of temperature over the range observed (10° to 60°C). The magnetization transfer rate was constant over the temperature range of 25° to 45°C, but deviated from this constant value at lower and higher temperatures. Additional measurements of diffusion coefficient in sphingomyelin were conducted. The diffusion coefficient was found to have a linear dependence as a function of temperature over the range observed (10° to 60°C). The temperature dependence between magnetization transfer and diffusion coefficient suggests that diffusion may be a rate-limiting factor in magnetization transfer over the physiological temperature range (25° to 45°C). The activation energy for the diffusion of water in water is 0.18 to 0.20 eV; for water in lipid systems, it is 0.28 eV; but for the magnetic transfer rate, the activation energy is 0.35 eV.

PROPOSED COURSE: No further studies on the interaction between diffusion coefficient and magnetization transfer rate as a function of temperature are expected. Dr. Balaban's group is working on a paper on this subject and will submit it in the near future.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10385-02 BEI
PERIOD COVERED October 1, 1992 to September 30, 1993		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Diffusion Tensor Echo-Planar Imaging		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) James Mattiello, Ph.D. Staff Fellow MES, BEIP, NCRR Peter J. Basser, Ph.D. Staff Scientist MES, BEIP, NCRR		
COOPERATING UNITS (if any) CE, NHLBI; DR, CC (D. LeBihan); DIR, NINDS		
LAB/BRANCH Biomedical Engineering and Instrumentation Program		
SECTION Mechanical Engineering Section		
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, MD 20892		
TOTAL STAFF YEARS: 0.6	PROFESSIONAL: 0.6	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) _(a) Human _(b) Human X (c) Neither subjects tissues _(a1) Minors _(a2) Interviews		
SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.) Echo-planar imaging (EPI) allows a magnetic resonance image to be acquired in less than 0.1 sec, which is orders of magnitude faster than existing MR modalities. This fast imaging technique allows us to monitor biological processes that evolve rapidly in time without observable motion artifacts, such as thermal conduction and molecular diffusion. We are extending the work of Dr. Robert Turner (formerly with the BEIP, NCRR, and now with the Laboratory of Cardiac Energetics, NHLBI) to develop this EPI diffusion system on the 4.7-T MRI system at the In Vivo NMR Research Center. Moreover, to account for the complex interactions between imaging and diffusion gradients applied in typical diffusion-weighted imaging pulse sequences, we have derived analytic expressions for "b-matrix" elements needed to estimate the effective self-diffusion tensor off-line.		

MAJOR FINDINGS: Problems with the existing 4.7-T hardware have been corrected so that EPI can now be performed. This correction has enabled our group to implement and demonstrate the clinical feasibility of Diffusion Tensor EPI (DTEPI). Diffusion-weighted echo-planar images can now be acquired. The b-matrices for generalized EPI diffusion-weighted images have now been calculated analytically by Dr. Mattiello, thus permitting rapid estimation of the diffusion tensor from the EPI DWI and, more importantly, giving the first quantitative assesment of the complex and multiple interactions between imaging and diffusion gradients in both the parallel and perpendicular directions. Shown below are the results of a control experiment in which diffusion ellipsoids were constructed for an isotropic water phantom. Their regularity, uniformity, and spherical shape validate the hardware improvements and calculation of the b-matrix. Similar data have been obtained from diffusion-weighted, spin-echo 2D-FT imaging pulse sequences for water, gel, and ex vivo cat brain (see "NMR Diffusion Imaging and Spectroscopy"). These images showed uniform diffusion ellipsoids for water and nonuniform diffusion ellipsoids for anisotropic regions in the cat brain, such as the corpus callosum.



Diffusion ellipsoid image for a water phantom constructed from several echo-planar diffusion-weighted images.

PROPOSED COURSE: This EPI pulse sequence has been adapted to the whole-body 4-T superconducting magnet in the Laboratory of Cardiac Energetics, NHLBI; human clinical studies are planned in the near future. Additionally, we are collecting EPI diffusion-weighted images on the 1.5-T clinical magnets, from which

diffusion tensor images and diffusion ellipsoid images can be generated in human subjects.

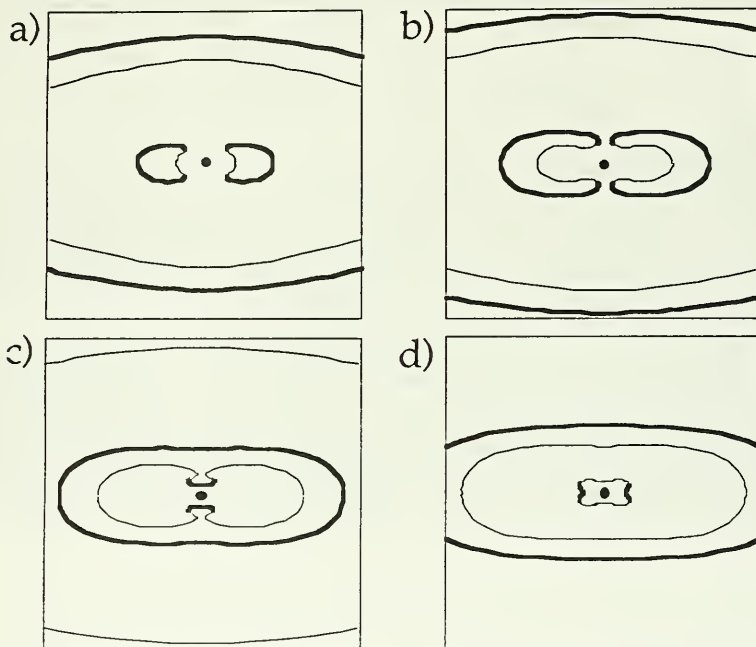
Temporal measurements of the diffusion coefficient, heated to measure the temperature variation within the phantom, are currently under way, using diffusion-weighted EPI.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10386-02 BEI
PERIOD COVERED October 1, 1992 to September 30, 1993		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Calculation of Electrical Activity in Cardiac Tissue		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Bradley J. Roth, Ph.D. Senior Staff Fellow MES, BEIP, NCRR		
COOPERATING UNITS (if any) LB, NINDS (J. Clay, V. Kowtha); Department of Biomedical Engineering, Duke University (N. Trayanova); Department of Physics & Astronomy, Vanderbilt University (J. Wikswo)		
LAB/BRANCH Biomedical Engineering and Instrumentation Program		
SECTION Mechanical Engineering Section		
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, MD 20892		
TOTAL STAFF YEARS: 0.15	PROFESSIONAL: 0.15	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews ☐ (b) Human tissues ☒ (c) Neither		
SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.) A numerical simulation has been performed of the propagation of an action potential through cardiac tissue. The bidomain model was used to account for the effect of both tissue anisotropy and the interstitial space on current flow. A relaxation technique was used to solve the nonlinear partial differential equations. The model has been modified to predict propagation in a two- or three-dimensional tissue due to a point source of current. The influence of unequal anisotropy ratios of the intracellular and extracellular conductivities is being investigated. Pairs of stimulus pulses are applied to a two-dimensional sheet of tissue to create rotors and other unusual phenomena, which could prove useful as models of cardiac arrhythmias and fibrillation. The interaction of cardiac tissue with a uniform electric field was also examined.		

OBJECTIVES: To increase our understanding of the electrical properties of cardiac tissue, and to provide a mathematical model that can be used to study cardiac arrhythmias.

SIGNIFICANCE: Cardiac arrhythmias and fibrillation are two of the most common causes of death among Americans. In order to understand these pathologies, a basic knowledge of the heart and the electrical properties of cardiac tissue is required. In this study we investigate idealized, yet important, models that reproduce many of the phenomena observed in cardiac tissue.

MAJOR FINDINGS: The intracellular and extracellular anisotropies influence the electrical properties of cardiac tissue. Tissues with "unequal anisotropy ratios" can lead to anodal stimulation and to arrhythmias generated by two pulses passing through a single electrode. Cardiac tissue is depolarized at the heart surface and deep within the tissue bulk by a uniform electric field. None of these phenomena could be observed without taking into account the different anisotropies inside and outside of the cells.



The wave front evoked by successive stimulation from a point source (represented by the dot). Frames a) through d) show the wave front at sequential instants. The thicker curve corresponds to the depolarization phase of the wave front, the thinner curve to the repolarization phase.

PROPOSED COURSE: The interaction of a spherical heart with a uniform electric field will be analyzed both analytically and numerically. The response of cardiac tissue to anodal stimulation will be examined, with an emphasis on explaining the "dip" in the anodal strength-interval curve.

PUBLICATIONS: Roth BJ. How the anisotropy of the intracellular and extracellular conductivities influences stimulation of cardiac muscle. J Math Biol 1992;30:633-46.

Saypol JM, Roth BJ. A mechanism for anisotropic reentry in electrically active tissue. J Cardiovasc Electrophysiol 1992;3:558-66.

Trayanova N, Roth BJ, Malden LJ. The response of a spherical heart to a uniform electric field: a bidomain analysis of cardiac stimulation. IEEE Trans Biomed Eng (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10387-02 BEI
PERIOD COVERED October 1, 1992 to September 30, 1993		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) A Three-Dimensional Motion Measurement System		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Horace E. Cascio, BSEE Electronics Engineer EEES, BEIP, NCRR		
COOPERATING UNITS (if any) BL, RM, CC (S. Stanhope); Adtech (A. Dainis)		
LAB/BRANCH Biomedical Engineering and Instrumentation Program		
SECTION Electrical and Electronic Engineering Section		
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, Maryland 20892		
TOTAL STAFF YEARS: 0.50	PROFESSIONAL: 0.40	OTHER: 0.10
CHECK APPROPRIATE BOX(ES) _(a) Human _(b) Human X (c) Neither subjects tissues _(a1) Minors (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The Biomechanics Laboratory makes quantitative information concerning human motion available to clinicians and researchers at the NIH. The BEIP has upgraded Dr. Stanhope's Vicon Motion and Image Analysis System by designing and fabricating six video-camera units, each with a variable intensity dual-wavelength infrared strobe. The upgrade solved many of the original Vicon system's problems, but fast motion analysis is still limited by the system's slow frame rate of 25 frames per second. The Biomechanics Laboratory requires a system to take data at 2000 frames per second. To satisfy this requirement, Dr. Stanhope requested that the BEIP develop a three-dimensional motion measurement system, using a concept patented by Dr. Andrew Dainis. The terms of the CRADA state that the NIH may develop a clinical operating 3-D motion analysis system using the concept; in return, the NIH may keep the system. Dr. Dainis will have rights to BEIP's refinements. In this venture with the Biomechanics Laboratory and Dr. Dainis, the BEIP will develop, fabricate, and test the system electronics. Dr. Dainis will develop the optics for the CCD linear arrays and the system software. The idea of Dr. Dainis's patent is that each camera has four linear CCD arrays, with each array having a specially designed lens. The arrays are placed on the face of the camera in a special pattern to translate a light source from a target into its spatial coordinates.		

OBJECTIVES: To design and fabricate a three-dimensional motion and image analysis system under a CRADA. The system will consist of several freestanding cameras, data acquisition and interfacing circuitry, and a controlling computer. A major goal of this project is to obtain a system with frame rates to 2000 Hz.

METHODS EMPLOYED: The system consists of one to sixteen cameras, a system controller, and a personal computer. The camera will contain signal processing, on-board memory, and interfacing circuitry to the system controller. The sensors are four 2048-element CCD linear arrays with a maximum sweep speed of 10 MHz. Frame rates near 5 KHz can be obtained, but a frame rate of 2 KHz would be acceptable. The cameras view targets located on a patient. The illumination of these targets can be active (LEDs or incandescent lamps) or passive (reflective spheres). A system controller will receive instructions from the host computer, and will then control the data acquisition session between all cameras. The cameras will be under computer control except when collecting data, when they will operate independently. The system controller releases the host computer for performing other real-time functions. A personal computer (PC) sets up the data acquisition procedures, then records real-time analog information from different sensors. At the completion of the testing session, the PC reads, analyzes, and displays the data from each camera in an appropriate form. For a multicamera system with requirements to collect position coordinates of many targets at a fast frame rate and to collect real-time analog information, the use of parallel CPUs adds to system efficiency and reliability. The 486 processor in the PC and a 186 processor on the embedded CPU card in the system controller give this system dual processing capability.

SIGNIFICANCE: A three-dimensional motion analysis system that can perform studies at high frame rates would be a welcome addition to the Biomechanics Laboratory, especially in studies of lip and tongue motions.

PROPOSED COURSE: To design and fabricate one camera unit and the System Control Unit. Work is currently proceeding in the design and fabrication of an active target and printed circuit boards required in the camera. The targets are battery-powered and, when triggered by an infrared light from a camera, will generate a high-energy infrared light back to the camera.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10388-02 BEI
PERIOD COVERED October 1, 1992 to September 30, 1993		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) 3-D NMR Imaging		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Ching-Nien Chen, Ph.D. Physical Scientist OD, BEIP, NCRR		
COOPERATING UNITS (if any) None		
LAB/BRANCH Biomedical Engineering and Instrumentation Program		
SECTION In Vivo NMR Research Center		
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, Maryland 20892		
TOTAL STAFF YEARS: 0.2	PROFESSIONAL: 0.2	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews ☐ (b) Human tissues ☒ (c) Neither		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) A new 3-D NMR imaging technique is under investigation. This technique requires a special set of magnetic field gradients to encode the 3-D information. A custom RF probe and gradients for a small test sample were constructed. Preliminary phantom studies have been completed. The initial analysis showed that the method is working as desired.		

PUBLICATION: Chen C-N, Hoult DI. The visualization of RF probe electric fields. Magn Reson Med 1993;29:386-90.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 RR 10389-02 BEI

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Data Processing of Fluorescence Microscope 3-D Images

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation)

Ching-Nien Chen, Ph.D. Physical Scientist OD, BEIP, NCRR

COOPERATING UNITS (if any)

Johns Hopkins University (S. Lesko)

LAB/BRANCH

Biomedical Engineering and Instrumentation Program

SECTION

In Vivo NMR Research Center

INSTITUTE AND LOCATION

NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

0.3

PROFESSIONAL:

0.3

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Fluorescence probes are designed and used to mark specific chromosomes in cells cultured from human fibroblasts. 3-D microscopic images obtained therefrom are used to study the spatial distribution of the chromosomes. They are generally regarded as randomly distributed in the cell. Images taken at certain stages of cell division are reoriented according to certain preset rules, then analyzed to see if there is any degree of order existent. Spatial orders, if found, may be significant to the understanding of cell division mechanisms.

METHODS EMPLOYED: Triple *in-situ* hybridization using chromosome-specific alpha satellite DNA probes was performed on human peripheral blood lymphocytes in order to determine the spatial arrangement of the centromeres of chromosomes 7, 11, and 17 in interphase nuclei. Three stacks of images were collected for each nucleus, using filter combinations for red, green, and blue fluorescence. The coordinates of the centromeres were then determined. Twenty-one sets of such data were obtained. The 21 sets of coordinates were transformed according to certain given rules, and the scatter of data was determined.

MAJOR FINDINGS: Preliminary studies showed that there is indeed some order and clustering for the three kinds of chromosomes probed (No. 7, No. 11, and No. 17). This finding may be significant to the understanding of cell division mechanisms with relation to the spatial distribution of chromosomes. A poster was presented at the 5th International Symposium and Workshops of the Society of Chinese Bioscientists in America, June 15-20, 1993, in Baltimore, Maryland. A manuscript entitled "Study of the Spatial Arrangement of Chromosomes 7, 11, and 17 Centromeres in Interphase Human Lymphocytes Using Digital Imaging Fluorescence Microscopy" is in preparation.

PROPOSED COURSE: We intend to probe and study more chromosomes (e.g., seven) to ascertain if the clustering of those we studied before is a general rule. We are also seeking better methods of cell fixing (or more rigid cells) so that deformation of cell shape will not contribute to errors in the determination of the coordinates.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10392-02 BEI												
PERIOD COVERED October 1, 1992 to September 30, 1993														
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Remote Laser Control for Photodynamic Therapy														
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">Thomas R. Clem, Sr., BSEE</td> <td style="width: 33%;">Electronic Engineer</td> <td style="width: 33%;">EEES, BEIP, NCRR</td> </tr> <tr> <td>Walter S. Friauf, MEE</td> <td>Section Chief</td> <td>EEES, BEIP, NCRR</td> </tr> <tr> <td>John Cole, MSEE</td> <td>Electronic Engineer</td> <td>EEES, BEIP, NCRR</td> </tr> <tr> <td>Paul D. Smith, Ph.D.</td> <td>Physicist</td> <td>EEES, BEIP, NCRR</td> </tr> </table>			Thomas R. Clem, Sr., BSEE	Electronic Engineer	EEES, BEIP, NCRR	Walter S. Friauf, MEE	Section Chief	EEES, BEIP, NCRR	John Cole, MSEE	Electronic Engineer	EEES, BEIP, NCRR	Paul D. Smith, Ph.D.	Physicist	EEES, BEIP, NCRR
Thomas R. Clem, Sr., BSEE	Electronic Engineer	EEES, BEIP, NCRR												
Walter S. Friauf, MEE	Section Chief	EEES, BEIP, NCRR												
John Cole, MSEE	Electronic Engineer	EEES, BEIP, NCRR												
Paul D. Smith, Ph.D.	Physicist	EEES, BEIP, NCRR												
COOPERATING UNITS (if any) ROB, DCT, NCI (A. Russo, T. Delaney); SB, DCT, NCI (H. Pass)														
LAB/BRANCH Biomedical Engineering and Instrumentation Program														
SECTION Electrical and Electronic Engineering Section														
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, Maryland 20892														
TOTAL STAFF YEARS: 0.2	PROFESSIONAL: 0.2	OTHER: 0.0												
CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%; vertical-align: top;"> ☒ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews </td> <td style="width: 33%; vertical-align: top;"> ☐ (b) Human tissues </td> <td style="width: 33%; vertical-align: top;"> ☐ (c) Neither </td> </tr> </table>			☒ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews	☐ (b) Human tissues	☐ (c) Neither									
☒ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews	☐ (b) Human tissues	☐ (c) Neither												
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Photodynamic therapy (PDT), which is the interaction of light with hematoporphyrin derivative (HPD or Photofrin II) in tissue, is in clinical use in several areas of application. Currently, PDT is being used clinically for bronchial obstructions, carcinoma in situ of the bladder, and intraperitoneal and pleural tumors. The treatment light is provided by two high-powered argon ion/dye lasers, each capable of operating at 514 nm or 630 nm, and at two delivery levels. Since the lasers have RS-232 interfaces for control and monitoring, a computer makes an ideal device for remote control from the operating room. The computer can also provide timing for each segment, a permanent log of level versus time, and integrated energy delivered, as well as allowing the individual operating the light-monitoring system to control the illumination.														

OBJECTIVE: To develop a computer-based remote-control system for the two lasers used for photodynamic therapy on human patients.

METHODS EMPLOYED: A small laptop computer is connected to each laser via a two-channel RS-232 interface box. A program has been written that allows this computer to control the functions of both lasers and to read the current status of various parameters from each laser. The program stores the laser powers at regular intervals, the time of each change in laser state, significant times and energy levels, and user-entered comments.

MAJOR FINDINGS: This system provides a very convenient and flexible method for controlling the laser energy levels from the operating room at the site of the procedure.

SIGNIFICANCE: Fewer personnel are now needed during a PDT procedure; the surgeon-requested changes in laser conditions are made much more quickly; the system operator in the operating room has a much clearer indication of what the lasers are doing at any time during a procedure; and there is a permanent record of both the status of each laser and the changes in operating conditions throughout the procedure.

PROPOSED COURSE: Further software developments will be made as new requirements arise during use for both current and new types of treatment.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 RR 10393-02 BEI

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Thermal Cycling of Incubator Baths for Polymerase Chain Reaction

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Thomas R. Clem, Sr., BSEE

Electronic Engineer

EEES, BEIP, NCRR

Paul D. Smith, Ph.D.

Physicist

EEES, BEIP, NCRR

COOPERATING UNITS (if any)

ROB, DCT, NCI (A. Russo)

LAB/BRANCH

Biomedical Engineering and Instrumentation Program

SECTION

Electrical and Electronic Engineering Section

INSTITUTE AND LOCATION

NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

0.5

PROFESSIONAL:

0.0

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human
subjects

☐ (b) Human
tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The polymerase chain reaction has rapidly become an indispensable tool of molecular biology. For new agents, searching for the most effective thermal cycle for lysis, quenching, and annealing of the nucleotide is a time-consuming process. The instrumentation being developed will streamline the search process by automatically cycling the reactants through predetermined temperature profiles. The technology employs PC-based computer control of five independent thermoelectric modules, and is readily expandable to greater numbers of reactant chambers.

OBJECTIVES: To develop thermal cycler instrumentation for searching and establishing the optimal conditions for new reagents used in the polymerase chain reaction. The instrumentation must perform independent ramp and soak temperature cycles for each of five reaction chambers.

METHODS EMPLOYED: Thermoelectric (Peltier) modules are capable of heating or cooling an element, depending on the direction of current flow through the module. The PCR reaction chamber, holding 200 μ l of reactant, is mounted in a copper housing to which two 19-watt thermoelectric modules are attached on opposite faces. The outer face of each module is attached to a water-cooled heat exchanger.

A typical thermal cycle calls for a temperature sequence of 50C to 90C to 70C and back to 50C. Ramping the temperature between these set points must be achieved in 30 seconds or less. The reaction chamber is held at each set point for times between 30 seconds and 2 minutes. Each set point must be reproducible in subsequent cycles and must be accurate to 1C. A prototype single-well setup showed that the thermoelectric modules easily achieved the ramping and set-point requirements, using an auxiliary water circulator at 50C for the heat exchanger.

Control of the thermal cycles will be achieved by a program running on a desktop PC. The program will use PID techniques to drive the thermal elements in a manner that will provide the required rapid temperature jump. The thermal elements will be driven by power-operational amplifiers, and the temperature will be measured by platinum RTD devices. The control program will allow selection of parameters for each well, and will display the current set-points for each active well and a plot of the progress of each.

SIGNIFICANCE: The polymerase chain reaction is an indispensable technique of molecular biology. This development will significantly reduce the time spent on searching for the optimum temperature cycle associated with new reagents as they become available. The concepts employed in this design are not limited to the prototype five-well system being developed, but are readily expandable to larger systems.

PROPOSED COURSE: To complete the design and construction of the interface hardware; to finish writing and to debug the control and data acquisition programs; to determine optimal PID constants; and to test and deliver the final operating system.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 RR 10394-02 BEI

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biological Pulsed Electronic Spin Resonance System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Thomas J. Pohida, MSEE	Electronic Engineer	EEES, BEIP, NCRR
Walter S. Friauf, MEE	Section Chief	EEES, BEIP, NCRR
Paul D. Smith, Ph.D.	Physicist	EEES, BEIP, NCRR

COOPERATING UNITS (if any)

ROB, NCI (J. Mitchell, A. Russo, J. Bourg, M. Krishna, S. Subramanian);
LCP, NIDDK (R. Tschudin); CSL, DCRT (H. Fredrickson)

LAB/BRANCH

Biomedical Engineering and Instrumentation Program

SECTION

Electrical and Electronic Engineering Section

INSTITUTE AND LOCATION

NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

1.4

PROFESSIONAL:

1.2

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects	☐ (b) Human tissues	☒ (c) Neither
☐ (a1) Minors		
☐ (a2) Interviews		

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

For several years, nitroxides have been a major focus of research in ROB, NCI because of their importance to radiation biology in general and their potential utility for new photodynamic therapy techniques. Electron spin resonance (ESR) is a powerful tool for free radical studies, but in the past it has been of limited value for biological work (especially *in vivo*) because of the excessive attenuation by water of the radio frequencies ordinarily used, about 9 GHz. The system now being developed will operate at about one-thirtieth of that frequency to alleviate the attenuation problem, and will incorporate pulse techniques developed in NMR, as well as other new techniques, to compensate for the inevitable thirtyfold loss of sensitivity.

OBJECTIVES: To develop a pulsed ESR apparatus optimized for the study of nitroxides and other compounds of interest under *in vivo* conditions. Initial measurements will be spectroscopic, but imaging will be subsequently undertaken.

METHODS EMPLOYED: The initial configuration will comprise a set of Helmholtz coils, a probe, a pulse programmer, a radio frequency system, data acquisition and processing circuitry, and a PC (or perhaps two PCs) for overall system control and data handling. New excitation techniques are under study to provide the broad bandwidth needed for nitroxides (approximately 100 MHz) without requiring excessive peak power. EEES personnel have designed and constructed a custom high-speed sampler/averager. Commercially available averaging units are not fast enough for these measurements--a limit imposed by the very short relaxation time of electrons compared to nuclei. This unit will sample and average 1000 free-induction decays, and will transfer the data to the computer in about 5 ms. The computer can further average 1000 such transfers, resulting in the averaging of 1,000,000 decays in about five seconds, with a thousandfold (or 60-decibel) improvement in signal-to-noise ratio, which will help compensate for the low sensitivity resulting from operation at a low frequency and other factors. In addition, EEES personnel have designed and constructed various radio-frequency components for the spectrometer. To enable averaging, for example, a multiple-frequency generator box was developed to provide a fixed-phase relationship (at each excitation) between all relevant frequencies. A narrowband transmit/receive (T/R) switch has also been delivered, although a broadband T/R switch will eventually be required. EEES has devised a method of constructing a broadband switch by introducing nontraditional transmission line impedance mismatches. Helmholtz coils have been designed and constructed to provide a uniform magnetic field throughout a volume (5 cm x 5 cm x 10 cm) in their center.

SIGNIFICANCE: Achievement of the proposed specifications will greatly facilitate research on nitroxides and other compounds of interest in biological systems.

PROPOSED COURSE: To implement the new broadband T/R switch. To design additional radio-frequency components as required by the spectrometer system. To provide complex signal processing algorithms for averaged data processing.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10395-02 BEI									
PERIOD COVERED October 1, 1992 to September 30, 1993											
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Digital Differential Thermistor Thermometer											
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">Walter S. Friauf, MEE</td> <td style="width: 33%;">Section Chief</td> <td style="width: 33%;">EEES, BEIP, NCRR</td> </tr> <tr> <td>Thomas R. Clem, Sr., BSEE</td> <td>Electronic Engineer</td> <td>EEES, BEIP, NCRR</td> </tr> </table>			Walter S. Friauf, MEE	Section Chief	EEES, BEIP, NCRR	Thomas R. Clem, Sr., BSEE	Electronic Engineer	EEES, BEIP, NCRR			
Walter S. Friauf, MEE	Section Chief	EEES, BEIP, NCRR									
Thomas R. Clem, Sr., BSEE	Electronic Engineer	EEES, BEIP, NCRR									
COOPERATING UNITS (if any) LBC, NHLBI (R. Berger)											
LAB/BRANCH Biomedical Engineering and Instrumentation Program											
SECTION Electrical and Electronic Engineering Section											
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, Maryland 20892											
TOTAL STAFF YEARS: 0.5	PROFESSIONAL: 0.4	OTHER: 0.1									
CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;"> ☐ (a) Human subjects </td> <td style="width: 33%;"> ☐ (b) Human tissues </td> <td style="width: 33%;"> ☒ (c) Neither </td> </tr> <tr> <td> ☐ (a1) Minors </td> <td colspan="2"></td> </tr> <tr> <td> ☐ (a2) Interviews </td> <td colspan="2"></td> </tr> </table>			☐ (a) Human subjects	☐ (b) Human tissues	☒ (c) Neither	☐ (a1) Minors			☐ (a2) Interviews		
☐ (a) Human subjects	☐ (b) Human tissues	☒ (c) Neither									
☐ (a1) Minors											
☐ (a2) Interviews											
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.) Full exploitation of the potential of differential calorimetry requires small sensors with rapid response and high sensitivity, virtually limiting the choice to thermistors. The small size is necessary so that the sensors can be very close together to avoid masking a minute difference in heat of reaction by extraneous temperature gradients. For convenient use by laboratory and health-care personnel, the apparatus must be fully automated.											

OBJECTIVES: To develop a fully automated differential thermistor bridge with high speed, sensitivity, and common mode rejection.

METHODS EMPLOYED: A personal computer (PC) with a standard plug-in interface board was used to provide the automation features, as well as data storage and display. A custom bridge configuration was designed to allow the computer to control balancing without the usual adjustment of the resistance of one arm, which is impossible with any standard interface board. The computer also controls bridge excitation to set the power level and allow sensing and correction of all thermoelectric potentials and amplifier offsets, as well as linear time variance of both.

SIGNIFICANCE: Achievement of the desired objectives will allow the construction and use of small probes with a suitable coating sensitive to the concentration of a specific compound on one thermistor. The instrument is also useful for general differential calorimetry, particularly when very fast response is needed.

PROPOSED COURSE: The instrument has been designed and built, and performs very near the theoretical limits of speed and sensitivity imposed by the Johnson noise of the thermistors. It is now undergoing evaluation in several experimental applications. The development of specific compound probes will be undertaken soon. Circuit analysis has disclosed that departure from perfect balance affects thermal common mode rejection, so that a slight imbalance can be used to compensate for imperfect matching of the temperature coefficients of the two thermistors. If necessary, the software needed to effect this imbalance will be developed. Software for several new operating modes has been developed, and a second unit with improved mechanical layout is under construction.

PUBLICATION: Friauf WS, Clem Sr TR, Sr., Berger RL. A personal computer-controlled differential thermistor bridge. In: Proceedings of the seventh international symposium on temperature. Amer Inst of Physics, 1992 (in press).

PATENT: A patent application has been filed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10396-02 BEI									
PERIOD COVERED October 1, 1992 to September 30, 1993											
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Characterization of Frozen-Hydrated Specimens by EELS											
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 35%;">Richard Leapman, Ph.D.</td> <td style="width: 35%;">Physical Scientist</td> <td style="width: 30%;">OD, BEIP, NCRR</td> </tr> <tr> <td>Stanley Sun, Ph.D.</td> <td>Visiting Fellow</td> <td>OD, BEIP, NCRR</td> </tr> <tr> <td>Shanling Shi, M.S.</td> <td>Visiting Associate</td> <td>OD, BEIP, NCRR</td> </tr> </table>			Richard Leapman, Ph.D.	Physical Scientist	OD, BEIP, NCRR	Stanley Sun, Ph.D.	Visiting Fellow	OD, BEIP, NCRR	Shanling Shi, M.S.	Visiting Associate	OD, BEIP, NCRR
Richard Leapman, Ph.D.	Physical Scientist	OD, BEIP, NCRR									
Stanley Sun, Ph.D.	Visiting Fellow	OD, BEIP, NCRR									
Shanling Shi, M.S.	Visiting Associate	OD, BEIP, NCRR									
COOPERATING UNITS (if any) LN, NINDS (S. B. Andrews); VRP, NCRR (W. Rall); Lehigh University (J. A. Hunt)											
LAB/BRANCH Biomedical Engineering and Instrumentation Program											
SECTION Electron Beam Imaging and Microspectroscopy Group											
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, MD 20892											
TOTAL STAFF YEARS: 1.0	PROFESSIONAL: 1.0	OTHER: 0.0									
CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">☐ (a) Human subjects</td> <td style="width: 33%;">☐ (b) Human tissues</td> <td style="width: 33%;">☒ (c) Neither</td> </tr> <tr> <td>☐ (a1) Minors</td> <td></td> <td></td> </tr> <tr> <td>☐ (a2) Interviews</td> <td></td> <td></td> </tr> </table>			☐ (a) Human subjects	☐ (b) Human tissues	☒ (c) Neither	☐ (a1) Minors			☐ (a2) Interviews		
☐ (a) Human subjects	☐ (b) Human tissues	☒ (c) Neither									
☐ (a1) Minors											
☐ (a2) Interviews											
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The technique originated by us last year for measuring water distributions in thin, frozen-hydrated biological specimens by means of electron energy loss spectroscopy (EELS) has been further developed. This approach depends on quantifying subtle changes in the valence electron excitation spectrum as a function of composition. It involves determining the single-scattering intensities, calculating oscillator strengths, and applying multiple least squares fitting to reference spectra for water and organic constituents. The technique has now been extended to spectrum-imaging, so that two-dimensional water distributions can be obtained from hydrated cryosections. Automated processing of the spectrum-image data has been implemented, and ways of reducing the computation time are being investigated. Applications of the technique (e.g., analyzing the water content of rapidly-frozen hepatocytes and cryopreserved embryos) are in progress.											

OBJECTIVES: To develop a direct method, based on electron energy loss spectroscopy, for obtaining quantitative water distributions at the subcellular level in frozen-hydrated tissue.

METHODS EMPLOYED: Hydrated tissue sections were cryotransferred into the BEIP scanning transmission electron microscope, where they were maintained at -180°C . By operating with reduced current into a defocused probe approximately 50 nm in diameter, EELS spectrum-images could be acquired with a PC486 computer at low electron dose with acquisition times of around 1000 s.

MAJOR FINDINGS: It has been shown that a detailed analysis of the shape of the valence electron energy loss spectra of frozen-hydrated cryosections provides a means of measuring water content. This technique depends on precisely fitting appropriate reference spectra for different components of the cell. The results indicate that the reference spectrum from protein alone may be sufficient to represent the organic constituents. Determination of the water content also requires knowledge of the ratio of inelastic cross sections per unit mass for water and protein. This quantity can be calculated by applying sum-rules for the differential oscillator strength, and also results in an estimate for the inelastic mean free path, which is necessary to estimate the specimen thickness. The technique promises to yield useful information about water distributions in subcellular compartments and could find application in physiological studies involving water regulation.

SIGNIFICANCE: The water content of compartments in the cell is an important physiological quantity that reflects how fluid and electrolyte concentrations are regulated, both indirectly by osmotic effects following ion movements and directly through water-specific channels. Changes in water content may accompany important cellular events such as secretion. In addition, changes in water distributions can indicate pathological states of the tissue, such as the swelling of neuroglia after brain injury. The new EELS technique potentially provides a means of obtaining direct water measurements in cells at below 100 nm spatial resolution for the first time.

PROPOSED COURSE: To accelerate the computer-processing time for analysis of the spectrum images by optimizing the software and hardware. To incorporate a faster EELS parallel-detector in order to decrease the acquisition time. To apply this new technique to various biological problems, including the water content of brain tissue and the characterization of water distributions in cryopreserved rodent embryos that are stored to maintain genetic strains for biomedical research.

PUBLICATIONS: Sun S, Shi S Leapman RD. Water distributions of hydrated biological specimens by valence electron energy loss spectroscopy. Ultramicroscopy 1993 (in press).

Shi S, Sun S, Andrews SB, Leapman RD. EELS measurement of hydrated cryosection thicknesss. Proceedings of the 27th annual meeting of the Microbeam Analysis Society. San Francisco: San Francisco Press, 1993 (in press).

Sun S, Shi S, Hunt JA, Leapman RD. Parallel-EELS mapping of water distributions in cryosectioned cells. Proceedings of the 27th annual meeting of the Microbeam Analysis Society. San Francisco: San Francisco Press, 1993 (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10397-02 BEI
PERIOD COVERED October 1, 1992 to September 30, 1993		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Subcellular Composition of the Pancreatic Islets of Langerhans		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Richard Leapman, Ph.D. Physical Scientist OD, BEIP, NCRR Shanling Shi, M.S. Visiting Associate OD, BEIP, NCRR Stanley Sun, Ph.D. Visiting Fellow OD, BEIP, NCRR		
COOPERATING UNITS (if any) LCBG, NIDDK (I. Atwater); SUNY-Stony Brook (M. Foster)		
LAB/BRANCH Biomedical Engineering and Instrumentation Program		
SECTION Electron Beam Imaging and Microspectroscopy Group		
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, MD 20892		
TOTAL STAFF YEARS: 0.2	PROFESSIONAL: 0.2	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews ☐ (b) Human tissues ☒ (c) Neither		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) We are continuing experiments to analyze rapidly frozen cultured rat islets of Langerhans at the level of individual secretory granules. Elemental analysis of thin, dried cryosections has shown that beta (or B) granules can be distinguished by high zinc, calcium, and sulfur, whereas non-beta (mainly alpha, or A) granules contain elevated phosphorus and magnesium. Although a single granule type predominated in a particular cell, some rebel granules were found in A cells that had the compositional fingerprint of B-cell granules. Zinc, which was found in millimolar concentrations in B-cell granules, was considered a marker for the insulin-storage complex. The data indicate that non-B islet cells in the adult pancreas may produce insulin-containing organelles and that, when glucagon and insulin are coexpressed, these hormones are packaged in separate granules. Efforts are now in progress to achieve better freezing conditions and improved quality of cryosections.		

OBJECTIVES: To measure the extent of compositional heterogeneity among secretory granules within individual cells and between cells of the pancreatic islet of Langerhans, and to gain information about relationships between cellular environment and granular structure and function.

METHODS EMPLOYED: Islets isolated from collagenase-digested rat pancreata were rapidly frozen against a liquid nitrogen-cooled copper block, mounted in a cryoultramicrotome and sectioned to a thickness of ~100 nm. Sections were deposited on carbon-coated grids and cryotransferred into either a conventional TEM-STEM or the BEIP field-emission STEM.

MAJOR FINDINGS: The distinctive compositions of alpha and beta granules have already indicated that glucagon and insulin are packaged into different granules. Improved specimen preparation techniques should provide more detailed information about the distributions of specific ions in these organelles. By modifying the cryoultramicrotome to incorporate an antistatic gun and a low-angle diamond knife, greatly improved cryosections have now been obtained from a number of different tissues.

SIGNIFICANCE: It has been believed that insulin biosynthesis in adult vertebrates is strictly confined to pancreatic B cells. Since B cells are selectively destroyed in some types of diabetes, it would be of clinical interest to control induction as well as secretion of insulin in non-B cells. The EM observation suggesting that beta-type granules may occur in A cells raises the important question of how hormones are sorted into separate secretory granules. One possible mechanism for separate packaging could involve specific "sorting domains" or three-dimensional epitopes on the insulin and glucagon prohormones.

PROPOSED COURSE: It is planned to obtain x-ray analytical data on cultured beta and alpha cells that can be manipulated biochemically *in vitro* when these preparations become available. Immunolabeling experiments are also planned at the level of the individual secretory granules in order to independently establish the identity of the islet cells.

PUBLICATIONS: Foster MC, Leapman RD, Li MX, Atwater I. Elemental composition of secretory granules in pancreatic islets of Langerhans. *Biophys J* 1993;64:525-32.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10398-02 BEI
PERIOD COVERED October 1, 1992 to September 30, 1993		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) NMR Diffusion Imaging and Spectroscopy		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation) Peter Basser, Ph.D. Senior Staff Fellow MES, BEIP, NCRR James Mattiello, Ph.D. Staff Fellow MES, BEIP, NCRR		
COOPERATING UNITS (If any) DR, CC (D. LeBihan)		
LAB/BRANCH Biomedical Engineering and Instrumentation Program		
SECTION Mechanical Engineering Section		
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, MD 20892		
TOTAL STAFF YEARS: 0.8	PROFESSIONAL: 0.8	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) _ (a) Human _ (b) Human X (c) Neither subjects tissues _ (a1) Minors _ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) We have related the diagonal and off-diagonal elements of the effective self-diffusion tensor, D , to the echo intensity in NMR spin-echo experiments. Using this expression, we have designed pulse sequences from which we can estimate D using a series of diffusion-weighted images. This estimate is validated using isotropic and anisotropic media. One application of this work is to use D to determine fiber orientation noninvasively <i>in vivo</i> . Moreover, we can infer mean diffusion distances and tissue microstructure within a voxel. We also have developed a new MR imaging modality: Diffusion Tensor MRI, in which we use D to construct images of diffusion ellipsoids that depict the local fiber orientation and mean diffusion distances, as well as images of the three scalar invariants of D that are independent of the reference frame in which magnetic field gradients and D are measured, and to provide new information about tissue microstructure and physiology. Clinical applications are anticipated in stroke monitoring and tumor diagnosis.		

OBJECTIVES: To increase our understanding of the microscopic milieu in various biological tissues from a knowledge of the displacement distribution of protons and metabolites, and to develop clinically feasible methods to measure them.

SIGNIFICANCE: No noninvasive method exists to measure proton diffusion *in vivo*, other than NMR. The potential value of measuring the effective diffusion tensor is in determining local changes in membrane permeability, monitoring edema, and determining local microstructure of tissue, such as its fiber orientation. In anisotropic tissue, like brain white matter and muscle, new quantities (e.g., the trace of the diffusion tensor) are proposed to replace the diffusion coefficient that might be measured and monitored in disease. This method may have applications in nondestructive testing, as well.

MAJOR FINDINGS: The effective diffusion tensor has been estimated for isotropic and anisotropic phantoms, water, and skeletal muscle tissue. We have successfully been able to infer fiber direction in skeletal muscle noninvasively to within less than one percent. Significant errors are made in diffusion NMR spectroscopy and imaging of anisotropic skeletal muscle when off-diagonal elements of $\mathbf{D}$ are not considered. We have successfully extended diffusion tensor MR spectroscopy to Diffusion Tensor MRI.

PROPOSED COURSE: To explore the benefits and uses of Diffusion Tensor MRI with a varied patient population.

PUBLICATIONS: LeBihan D, Basser PJ, Mattiello J, Cuenod CA, Posse S, Turner R. Assessment of NMR diffusion measurements in biological systems: effects of microdynamics and microstructure. In: SMRM workshop: functional MRI of the brain. Arlington: SMRM, 1993;19-26.

Basser PJ, Mattiello J, LeBihan D. The effective self-diffusion tensor: its relationship to and estimation from the NMR spin-echo signal. *Journal of Magnetic Resonance Series B* 1994 (in press).

Basser PJ, LeBihan D. Diffusion anisotropy measured using NMR. In: LeBihan D, Rosen B, eds. *Diffusion imaging in radiology*. New York: Raven Press (in press).

Mattiello J, Basser PJ, LeBihan D. Analytic expressions for the $\mathbf{b}$ matrix used in diffusion imaging. In: LeBihan D, Rosen B, eds. *Diffusion imaging in radiology*. New York: Raven Press (in press).

LeBihan D, Basser PJ, Mattiello J, Cuenod CA, Posse S, Turner R. Assessment of NMR diffusion measurements in biological systems: effects of microdynamics and microstructure. In: LeBihan D, Rosen B, eds. *Diffusion imaging in radiology*. New York: Raven Press (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10399-02 BEI									
PERIOD COVERED October 1, 1992 to September 30, 1993											
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Development of a Cell Sorter											
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 40%;">John I. Peterson, Ph.D.</td> <td style="width: 30%;">Chemist</td> <td style="width: 30%;">CHES, BEIP, NCRR</td> </tr> <tr> <td>Stephen B. Leighton, Ph.D.</td> <td>Mechanical Engineer</td> <td>MES, BEIP, NCRR</td> </tr> <tr> <td>Thomas R. Clem, Sr., BSEE</td> <td>Electronics Engineer</td> <td>EEES, BEIP, NCRR</td> </tr> </table>			John I. Peterson, Ph.D.	Chemist	CHES, BEIP, NCRR	Stephen B. Leighton, Ph.D.	Mechanical Engineer	MES, BEIP, NCRR	Thomas R. Clem, Sr., BSEE	Electronics Engineer	EEES, BEIP, NCRR
John I. Peterson, Ph.D.	Chemist	CHES, BEIP, NCRR									
Stephen B. Leighton, Ph.D.	Mechanical Engineer	MES, BEIP, NCRR									
Thomas R. Clem, Sr., BSEE	Electronics Engineer	EEES, BEIP, NCRR									
COOPERATING UNITS (if any) MET, DCBDC, NCI (J. Berzofsky)											
LAB/BRANCH Biomedical Engineering and Instrumentation Program											
SECTION Chemical Engineering Section											
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, MD 20892											
TOTAL STAFF YEARS: 0.5	PROFESSIONAL: 0.5	OTHER: 0.0									
CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">_ (a) Human subjects</td> <td style="width: 33%;">_ (b) Human tissues</td> <td style="width: 33%;">X (c) Neither</td> </tr> <tr> <td>_ (a1) Minors</td> <td></td> <td></td> </tr> <tr> <td>_ (a2) Interviews</td> <td></td> <td></td> </tr> </table>			_ (a) Human subjects	_ (b) Human tissues	X (c) Neither	_ (a1) Minors			_ (a2) Interviews		
_ (a) Human subjects	_ (b) Human tissues	X (c) Neither									
_ (a1) Minors											
_ (a2) Interviews											
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) An instrument is being developed for selecting individual cells from growth medium and placing them in wells in a tray (or some other container) for testing or monoclonal growth. Traditional pipetting of a diluted cell suspension is slow and labor-intensive, and requires further processing to select the samples containing a single cell. Recently, a commercial instrument has become available (Quixell) that attaches to a microscope and consists essentially of a robotic pipetting system. This instrument has certain manipulative advantages, but requires visual cell selection with manual control. The instrument under development would combine automatic cell selection with computer-controlled cell placement.											

OBJECTIVES: The objective of this project is to develop an instrument that can select single cells from a growth medium and place them into wells on a tray.

METHODS EMPLOYED: The first approach is to test the feasibility of electrochemical detection of cells.

SIGNIFICANCE: The selection of single cells by pipetting from a very dilute medium is slow and tedious (although automatable), and is based on a statistical expectation of getting mostly single cells placed into wells. Further testing and evaluation must be done to discard wells with either no cells or more than one cell. The instrument envisioned would be completely automatic in cell selection and placement and would be of considerably greater value than the commercially available instrument, which places cells robotically but requires visual-manual selection.

MAJOR FINDINGS: The characteristics of a cell detection system were measured, based on the change of resistance of orifices during cell transport through them (the "Coulter-counter" principle). Cells and plastic microspheres were tested with sapphire orifices.

This system does not appear attractive for two reasons: 1) The electrical noise level is a problem. It might be possible to contend with this noise by using sophisticated noise-reduction techniques, but the timing and electrochemical cell construction requirements for cell separation would make it difficult. 2) The orifice technique requires optimization for a narrow range of cell size. A system designed for large cells (e.g., 20 micrometers in diameter) will have poor sensitivity of detection for smaller ones (e.g., 5 micrometers in diameter), and an orifice that is optimal for small cells will experience plugging problems with larger ones. A reasonably close match between cell size and orifice size is needed to produce an acceptable signal-to-noise ratio.

PROPOSED COURSE: The project will be pursued with the investigation of optical approaches. The basic problem is how to devise a system that does not depend on the cells' being dyed or otherwise altered.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10401-02 BEI						
PERIOD COVERED October 1, 1992 to September 30, 1993								
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Multiresolution Techniques for the Compression of X-Rays								
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">Michael Unser, Ph.D.</td> <td style="width: 33%;">Visiting Scientist</td> <td style="width: 33%;">OD, BEIP, NCRR</td> </tr> <tr> <td>Akram Aldroubi, Ph.D.</td> <td>Senior Staff Fellow</td> <td>OD, BEIP, NCRR</td> </tr> </table>			Michael Unser, Ph.D.	Visiting Scientist	OD, BEIP, NCRR	Akram Aldroubi, Ph.D.	Senior Staff Fellow	OD, BEIP, NCRR
Michael Unser, Ph.D.	Visiting Scientist	OD, BEIP, NCRR						
Akram Aldroubi, Ph.D.	Senior Staff Fellow	OD, BEIP, NCRR						
COOPERATING UNITS (if any) CEB, NLM (L. Berman, R. Long)								
LAB/BRANCH Biomedical Engineering and Instrumentation Program								
SECTION Office of the Director								
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, MD 20892								
TOTAL STAFF YEARS: 0.5	PROFESSIONAL: 0.5	OTHER: 0.0						
CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;"> ☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews </td> <td style="width: 33%;"> ☐ (b) Human tissues </td> <td style="width: 33%;"> ☒ (c) Neither </td> </tr> </table>			☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews	☐ (b) Human tissues	☒ (c) Neither			
☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews	☐ (b) Human tissues	☒ (c) Neither						
SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.) The purpose of this project is to develop new compression techniques and to study their efficiency for the coding of cervical and lumbar spine x-ray data. We have chosen to concentrate our efforts on multiresolution schemes, which are especially suited for progressive transmission. Our present approach uses a pyramidal image representation that consists of a series of increasingly coarser copies of the original, with a scale reduction by a factor of two at each step. The quantized differences between successive levels in this pyramid are then coded for transmission. The image is reconstructed in a progressive fashion, starting from its coarsest representation. This multiresolution decomposition is based on a least squares principle. It is, in fact, closely related to the wavelet transform. Our most recent contribution has been in the specification of optimal reduction filters for the representation of images with minimum loss of information.								

OBJECTIVES: There are currently four main objectives to this research: (1) the design of good filter banks for pyramid and wavelet decomposition; (2) the investigation of optimal methods for bit allocation and quantization; (3) the implementation of these algorithms on a UNIX workstation; and (4) comparison with other existing methods (e.g., JPEG cosine transform coding, vector quantization) using x-ray data. The final results will be evaluated by radiologists.

METHODS EMPLOYED: An image is represented by a pyramid in which each level is a coarser (and more compact) copy of the original. The pyramid and wavelet filters are designed to minimize the energy of the difference between two successive levels. These difference images are then quantized and represented by a reduced number of bits. This information is transmitted to the decoder, and the image is reconstructed progressively, starting from the coarsest representation. The least squares pyramid decomposition algorithm has been coded in C. These algorithms will be tested using the DXPnet database, which consists of a large collection of digitized x-rays of the cervical and lumbar spine.

MAJOR FINDINGS: Our preliminary results indicate that our least squares design technique provides a substantial performance improvement over the Laplacian/Gaussian pyramid commonly used in this context.

SIGNIFICANCE: These data compression techniques may turn out to be an essential component for the DXPnet project (digital x-ray prototype network). This project is sponsored by NLM, in collaboration with both the National Center for Health Statistics and the NIAMS. This study requires the transmission of a large number of x-rays over *Internet* to radiologists for evaluation. Currently, the transmission of a single noncoded image takes about 20 minutes; it is essential to reduce this time lag to a more acceptable level.

PROPOSED COURSE: The next step is to address the important issues of bit allocation and quantization.

PUBLICATIONS: Unser M. On the optimality of ideal filters for pyramid and wavelet signal approximation. IEEE Trans Signal Processing's special issue on wavelets (in press).

Unser M. An extension of the Karhunen-Loève transform for wavelets and perfect reconstruction filterbanks. In: Proceedings of the SPIE, Vol. 2034: Wavelet applications in signal and image processing (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10403-02 BEI									
PERIOD COVERED October 1, 1992 to September 30, 1993											
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Functional Analysis and Applications to Biomedical Image Processing											
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 40%;">Akram Aldroubi, Ph.D.</td> <td style="width: 30%;">Senior Staff Fellow</td> <td style="width: 30%;">OD, BEIP, NCRR</td> </tr> <tr> <td>Michael Unser, Ph.D.</td> <td>Visiting Fellow</td> <td>OD, BEIP, NCRR</td> </tr> <tr> <td>Murray Eden, Ph.D.</td> <td>Director</td> <td>OD, BEIP, NCRR</td> </tr> </table>			Akram Aldroubi, Ph.D.	Senior Staff Fellow	OD, BEIP, NCRR	Michael Unser, Ph.D.	Visiting Fellow	OD, BEIP, NCRR	Murray Eden, Ph.D.	Director	OD, BEIP, NCRR
Akram Aldroubi, Ph.D.	Senior Staff Fellow	OD, BEIP, NCRR									
Michael Unser, Ph.D.	Visiting Fellow	OD, BEIP, NCRR									
Murray Eden, Ph.D.	Director	OD, BEIP, NCRR									
COOPERATING UNITS (if any) None											
LAB/BRANCH Biomedical Engineering and Instrumentation Program											
SECTION Office of the Director											
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, Maryland 20892											
TOTAL STAFF YEARS: 1.0	PROFESSIONAL: 1.0	OTHER: 0.0									
CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">_ (a) Human subjects</td> <td style="width: 33%;">_ (b) Human tissues</td> <td style="width: 33%;">X (c) Neither</td> </tr> <tr> <td colspan="3">_ (a1) Minors</td> </tr> <tr> <td colspan="3">_ (a2) Interviews</td> </tr> </table>			_ (a) Human subjects	_ (b) Human tissues	X (c) Neither	_ (a1) Minors			_ (a2) Interviews		
_ (a) Human subjects	_ (b) Human tissues	X (c) Neither									
_ (a1) Minors											
_ (a2) Interviews											
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) An important aspect of biomedical data and image processing is to find signal representations that are adapted to the application. The Fourier transform, for example, is well adapted to analyzing the frequency components of signals. Shannon's sampling theory is well adapted to analogue digital conversions. Thus, it is well suited to the processing and storage of signals and images by digital computers. However, Shannon's sampling theory is restricted to bandlimited functions. We have developed a general sampling procedure for non-bandlimited signals. In particular, by considering sampling as a problem of approximation in translation-invariant function spaces, we have shown that the least squares approximation of a signal consists of a sampling procedure generalizing the classical one. It consists of an optimal prefiltering, a pure jitter-stable sampling, and a postfiltering for the reconstruction. We have also developed a general theory of representation that uses the new concepts of multiresolutions and wavelets, which are well adapted to multiscale signal processing, edge detection tasks, signal analyses, coding, and compression.											

OBJECTIVES: Our goal is to develop transforms that are well suited to different tasks of data processing and biomedical image processing. These transforms should be adapted to problems in signal analysis, such as EEG or ECG signals. They should also be useful for detection tasks and for storage and retrieval of large volumes of data, such as MRI images, image series, and 3-D images.

METHODS EMPLOYED: The sampling theory is based on the study of function spaces obtained by weighted sums of a generating function and its translations on the integers. The orthogonal projection of a signal on these spaces is equivalent to a sampling procedure, which consists of a prefiltering followed by a sampling and a postfiltering for the reconstruction. The multiresolution and wavelet theories of representation are similar to the sampling theory in that they are defined using a single function. The linear combination of the translations and dilations of the scaling function generates the multiresolution signal spaces. A similar construction with a wavelet function generates the wavelet spaces. By using convolution of scaling functions, we obtain other scaling functions with more smoothness. Using linear combination of the translations of a scaling function (or wavelet), we construct other scaling functions (or wavelets) with certain desired properties.

MAJOR FINDINGS: The general sampling theory we have derived generalizes Shannon's sampling procedure to non-bandlimited signal spaces. Moreover, we have established the link with Shannon's sampling theory. The convolution sequences of generating functions indexed with n allow us to construct sequences of increasingly smooth signal spaces. We have proven that the corresponding sampling schemes converge to the classical sampling procedure of Shannon as the index n tends to infinity. We have found a simple constructive method for constructing scaling and wavelet functions with some desired properties. For example, we can construct symmetrical interpolating wavelets that are as smooth as we want, and that have as good bandpass characteristics as we choose. We can also construct wavelets with optimal time-frequency localization, starting from any given wavelet function.

SIGNIFICANCE: This work will provide the scientific community with new tools for the analysis and processing of data and images. The sampling theory has already been applied to the design of new tomographic reconstruction algorithms. It has also been applied to the enlargement and reduction of images. The multiresolution and wavelet representations are currently being applied at NIH to image coding, image transmission, and the rotational and translational alignment of autoradiograms.

PUBLICATIONS: Aldroubi A, Unser M. Multiresolutions and wavelets: algebraic structure and families in relation with Shannon's sampling theory and the Gabor transform. In: Fossum RM, ed. 1992 annual meeting of the American Mathematical Society. Baltimore: 1992;13:82.

Aldroubi A, Unser M, Eden M. Cardinal spline filters: stability and convergence to the ideal sinc interpolator. Signal Processing 1992;28:127-38.

Aldroubi A, Unser M. Sampling procedures in function spaces and asymptotic equivalence with Shannon's sampling theory. Numerical Functional Analysis and Optimization (in press).

Aldroubi A, Unser M, Eden M. Discrete spline filters for multiresolutions and wavelets of l_2 . SIAM J Math Analysis (in press).

Aldroubi A. Portraits of frames: overcomplete representations with applications to image processing. In: Laine A, ed. 1993 SPIE-mathematical imaging: wavelet applications in signal and image processing (in press).

Aldroubi A, Unser M. Generalized sampling theory and application to multiresolutions and wavelets of L_2 . In: Laine A, ed. 1993 SPIE-mathematical imaging: wavelet applications in signal and image processing (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10404-02 BEI
PERIOD COVERED October 1, 1992 to September 30, 1993		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Mathematical Methods in Gel Electrophoresis		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Akram Aldroubi, Ph.D. Senior Staff Fellow OD, BEIP, NCRR Michael Unser, Ph.D. Visiting Fellow OD, BEIP, NCRR		
COOPERATING UNITS (if any) LTPB, NICHD (M. Garner, A. Chrambach); DCRT		
LAB/BRANCH Biomedical Engineering and Instrumentation Program		
SECTION Office of the Director		
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, Maryland 20892		
TOTAL STAFF YEARS: 2.0	PROFESSIONAL: 2.0	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) _(a) Human _(b) Human X (c) Neither subjects tissues _(a1) Minors _(a2) Interviews		
SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.) Electrophoresis is a technique widely used in biology to separate, classify, and study complex mixtures of macromolecules and particles (such as DNA). We have applied mathematical methods and techniques to problems in electrophoresis. In particular, we have developed models describing the motion of macromolecules and virus-sized particles in gels. We have defined a resolution function that is well adapted to electrophoretic separation of particles such as DNA. The models of motion, together with the resolution function, allow us to predict the minimal time and optimal migration path for the resolution of complex mixtures, such as those encountered in DNA sequencing. We have also developed methods for evaluating the efficiency of various polymers in the separation of macromolecules of various sizes. For this purpose, we have defined the separation efficiency function, as well as the resolution efficiency function. These functions are used to evaluate different polymers relative to one another. These techniques give us a practical means by which to select the most efficient sieving polymer solution for a particular molecular size. Finally, we have implemented the mathematical algorithms used in the analyses in a user-friendly software package for use on a personal computer.		

OBJECTIVES: Our goal is to develop models, techniques, and methods for the quantitative analysis of gel electrophoresis. More specifically, we want to develop means by which to predict the way different particles separate in various media.

METHODS EMPLOYED: The motion of the particles is described by combining Ogston's model of gel electrophoresis with the diffusion equation in partial differential equations. The resolution function is derived from a property of Gaussian functions. The time and migration path of particles in gels are deduced by combining the equations of motion with the resolution function. The efficiency function is deduced by using Ogston's model of particle mobility in gels for two particles with different radii, and letting the difference tend to zero.

MAJOR FINDINGS: Our models describe the motion of macromolecules and virus-sized particles in gels. The resolution function we have defined describes the degree of separation of a mixture of particle populations. The equations we derived predict the time length needed for separating a mixture under experimental conditions. The resolution efficiency function we have found allows us to compare the effectiveness of different media in sieving both large and small particles. Our theory has been applied to calculate the capillary electrophoresis of DNA sequencing separations. It showed that acceptable resolution could be obtained using shorter times than those commonly used in practice.

SIGNIFICANCE: A savings of time (by a factor of about 2.5) can be achieved in the separation of DNA mixtures. Thus, with our method, we can minimize the electrophoresis time in DNA sequencing, circumventing one of the limiting steps in electrophoresis. We have also applied our theory to evaluate four polymers relative to one another in terms of their efficiency in the separation of macromolecules of various sizes, and derived a nomogram that allows the practical selection of the sieving media and the conditions for the separation of molecules of a desired size range.

PUBLICATIONS: Aldroubi A, Garner M. Minimal electrophoresis time for DNA sequencing. *BioTechniques* 1992;13:620-4.

Tietz D, Aldroubi A, Pulyaeva H, Guszczyński T, Garner M, Chrambach A. Advances in DNA electrophoresis in polymer solutions. *Electrophoresis* 1992;13:614-16.

Chrambach A, Aldroubi A. The relative efficiency of molecular sieving in solutions of four polymers. *Electrophoresis* 1993;14:18-22.

OBJECTIVES: To improve the resolution of PET scans, it is necessary either to eliminate head motion or to correct for it. Since methods to eliminate head motion have been inadequate to date, the present system has been designed to measure head motion so that corrections can be made to the scan.

SIGNIFICANCE: The effective resolution of PET scans (~5 mm) is degraded by approximately a factor of 2, due to the effects of head motion during a scan and between two scans taken an hour apart. Elimination of this additional uncertainty in measurements would represent a significant advance. The cost of the system under development is only a few percent of the scanner's cost. Its use should put a minimal additional burden on the system operator, since it is under the control of a user-friendly computer.

METHODS EMPLOYED: Patient head motion is determined by optically measuring the positions of three blinking miniature lamps fixed to the head, using two position-sensitive detectors (PSDs). These devices sense the x- and y-coordinates of the centroid of the light falling on their photosensitive surface. By using sequentially blinking, extremely bright lights under stable, relatively dark ambient conditions, the PSD output can be related unambiguously to the lamp position. The sensor electronics is under GPIB control, which in turn is controlled by a Macintosh computer running the program Labview. This program does the required computations to generate the three angles and three positions required to specify head motion. The computer also controls the instrument setup, thus relieving the technician of this burden.

PROPOSED COURSE: The system will be installed shortly. It will then be evaluated and modified as necessary.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10406-02 BEI									
PERIOD COVERED October 1, 1992 to September 30, 1993											
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Development of a Fiber-Optic Gastric pH Sensor											
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">John I. Peterson, Ph.D.</td> <td style="width: 33%;">Chemist</td> <td style="width: 33%;">CHES, BEIP, NCRR</td> </tr> <tr> <td>Eden Netto, M.Sc.</td> <td>Electrical Engineer</td> <td>CHES, BEIP, NCRR</td> </tr> </table>			John I. Peterson, Ph.D.	Chemist	CHES, BEIP, NCRR	Eden Netto, M.Sc.	Electrical Engineer	CHES, BEIP, NCRR			
John I. Peterson, Ph.D.	Chemist	CHES, BEIP, NCRR									
Eden Netto, M.Sc.	Electrical Engineer	CHES, BEIP, NCRR									
COOPERATING UNITS (if any) None											
LAB/BRANCH Biomedical Engineering and Instrumentation Program											
SECTION Chemical Engineering Section											
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, MD 20892											
TOTAL STAFF YEARS: 1.5	PROFESSIONAL: 1.5	OTHER: 0.0									
CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">X (a) Human subjects</td> <td style="width: 33%;">(b) Human tissues</td> <td style="width: 33%;">(c) Neither</td> </tr> <tr> <td>(al) Minors</td> <td></td> <td></td> </tr> <tr> <td>(a2) Interviews</td> <td></td> <td></td> </tr> </table>			X (a) Human subjects	(b) Human tissues	(c) Neither	(al) Minors			(a2) Interviews		
X (a) Human subjects	(b) Human tissues	(c) Neither									
(al) Minors											
(a2) Interviews											
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.) A fiber-optic pH sensor, as well as associated instrumentation, is being developed for measurement and monitoring of intraesophageal and intragastric pH. Composed of replaceable sensors, an optical system, and electronic circuitry, the instrument should be able to measure pH in the range of 0-7, with an accuracy of 0.1 pH units and a response time in the range of 30 sec, for up to 24 hours. This instrument should be useful for the research, diagnosis, and evaluation of acid-related disorders of the upper gastrointestinal tract, and for studies on the influence of diet and antisecretory drugs.											

OBJECTIVES: The objective of this work is to develop a dye-based sensor that covers a broad pH range from neutral to high gastric acidities. Several fiber-optic pH sensor systems have been developed, based on extensions of the approach originated here by Drs. Peterson and Goldstein, but with one exception they have not been for the low pH range. (The exception is a system intended for very high acidity measurements in nuclear fuel technology.)

METHODS EMPLOYED: The project consists of several areas of work:

1. A system of dyes, having the correct optical and chemical characteristics (acidity constants) to cover the pH range of interest and to provide suitable wavelength and absorbance properties, must be developed and optimized.

2. This system must be combined with a dye support system having several intrinsic goals: to fix the dyes, so they will be stable; to provide a diffusion medium, so they will equilibrate with the surrounding pH; and to be formed into a probe system with a short response time. The possible toxicity of both the dye and the support system must be assessed.

3. A probe arrangement must be devised that will both be physically strong and have a container for the dye system that allows diffusion of hydrogen ions while limiting the flow of substances with higher molecular weights. Possible container materials must be tested.

4. Model instrumentation must be developed that involves experimentation to combine an optical system with the probe and the dye system. An instrument must be constructed for further testing of the performance of the pH probes. This step includes combining the optical system with electronic interfacing and computer programming.

5. When a working system is available, further *in vitro* testing and evaluation can be done for dye-system optimization and testing of probe stability, accuracy, and precision. Testing of the probe in gastric material will be necessary to discover potential problems, such as interference from colored substances and possible destructive action on the probe.

6. If the *in vitro* testing does not show major problems, then *in vivo* testing in animals should proceed to ensure acceptable performance. This testing would be done in conjunction with electrode measurements for comparison. Some testing in humans would be desirable, if possible.

SIGNIFICANCE: This project represents a potential for significant contribution to biomedical engineering and clinical research, since current techniques involving electrodes are uncomfortable, cumbersome, and subject to difficulties due to electrode coatings and electrical safety hazards. A suitably

designed fiber-optic probe would be smaller, safer, and more reliable, and would facilitate diagnostic and research measurements with a patient-wearable system.

MAJOR FINDINGS: The first three areas of work listed above have been developed; probes were made and evaluated for their optical performance and response to test solutions of various acidities. Combinations of dyes and a polymeric dye-support system, along with a method of probe construction using ion-permeable tubing, were developed. The latter part of the year was devoted primarily to developing suitable instrumentation that combines the probes with an optical and computer-operated system.

PROPOSED COURSE: Work is planned to evaluate the instrumentation and probe system *in vitro* and *in vivo*. This evaluation process will probably include further work to optimize the probe construction. Continuation of the work will depend on obtaining support for Mr. Netto, who has worked with BEIP on a scholarship from the Brazilian overnment.

PUBLICATIONS: Peterson JI, Netto EJ. Design considerations in making a broad-range fiber-optic pH sensor. In: Milanovitch FP, ed. Fiber-optic sensors in medical diagnostics: proceedings of the SPIE. Los Angeles: The Society of Photo-Optical Instrumentation Engineers, 1993;1886:98-108.

Netto EJ, Peterson JI, Wang B. Fiber-optic pH sensor for gastric measurements--preliminary results. In: Milanovitch FP, ed. Fiber-optic sensors in medical diagnostics: proceedings of the SPIE. Los Angeles: The Society of Photo-Optical Instrumentation Engineers, 1993;1886:109-17.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10407-02 BEI
PERIOD COVERED October 1, 1992 to September 30, 1993		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Elasticity and Active Force Generation in Cochlear Outer Hair Cells		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Richard Chadwick, Ph.D. Head, Theor. Biomech. Group OD, BEIP, NCRR		
COOPERATING UNITS (if any) LCB, NIDCD (K. Iwasa)		
LAB/BRANCH Biomedical Engineering and Instrumentation Program		
SECTION Office of the Director		
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, MD 20892		
TOTAL STAFF YEARS: 0.3	PROFESSIONAL: 0.3	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews ☐ (b) Human tissues ☒ (c) Neither		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) To establish the magnitude of the electromechanical force generated by the outer hair cell from the mammalian cochlea, we use a cylindrical membrane model characterized by area and shear moduli for a passive elastic element, and a membrane potential-dependent, active tension-generating element. We measured pressure-strain relations to determine the elastic moduli, and found the area modulus was both close to a lipid bilayer and an order of magnitude larger than the shear modulus. We also determined that the active tension element is nearly isotropic, with an amplitude sensitivity of about $2 \times 10^{-2} \text{ Nm}^{-1} \text{ V}^{-1}$. At 73 dB of acoustical stimulation, the active force generated per outer hair cell is about 0.6 nN--close to the force applied to a corresponding area of the basilar membrane by acoustic pressure. This finding supports the hypothesis that the outer hair cell acts as a feedback motor in the fine-tuning mechanism of the mammalian ear.		

OBJECTIVES: The motility of the outer hair cell (OHC) from the mammalian cochlea is considered essential for fine tuning. Extensive efforts have been directed toward characterizing the motility mechanism. It has been established that it is membrane potential-dependent. A hyperpolarizing pulse elongates the cell, and a depolarizing pulse shortens it. Tight-sealed membrane patches, formed on the lateral wall, undergo area changes when the membrane potential is changed. A hyperpolarizing pulse increases membrane area, and a depolarizing pulse reduces it. Although these electrokinetic shape changes are well characterized, it has not been previously demonstrated that an OHC can generate sufficient force to influence the basilar membrane. We use a biomechanical membrane model of the OHC to investigate this issue.

METHODS EMPLOYED: Isolated outer hair cells from guinea pig cochlea, light microscopy, inflation by micropipette, osmotic swelling, mathematical modeling, and image analysis are used in this project.

SIGNIFICANCE: The magnitude of the active force is comparable to that due to acoustic loading. Hence, the OHC has sufficient strength to act as a feedback motor for fine tuning of the mammalian cochlea.

PROPOSED COURSE: Capacitance measurements are being carried out to further assess the magnitude of OHC surface area changes. Experiments are planned to characterize the dynamic mechanical response of the OHC.

PUBLICATIONS: Iwasa KH, Chadwick RS. Elasticity and active force generation in cochlear outer hair cells. J Acoust Soc Am 1992;92:3169-73.

Iwasa KH, Chadwick RS. Factors influencing the length change of an auditory outer hair cell in a tight-fitting capillary. J Acoust Soc Am 1993;94 (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE		PROJECT NUMBER
NOTICE OF INTRAMURAL RESEARCH PROJECT		Z01 RR 10408-02 BEI
PERIOD COVERED October 1, 1992 to September 30, 1993		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Perfusion System for Animals		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Elijah C. Walker, M.S. Chief ACES, BEIP, NCRR		
COOPERATING UNITS (if any) SB, DCT, NCI (A. Thom, D. Fraker, J. Norton)		
LAB/BRANCH Biomedical Engineering and Instrumentation Program		
SECTION Applied Clinical Engineering Section		
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, Maryland 20892		
TOTAL STAFF YEARS: 1.5	PROFESSIONAL: 1.0	OTHER: 0.5
CHECK APPROPRIATE BOXES) _ (a) Human subjects _ (a1) Minors _ (a2) Interviews _ (b) Human tissues X (c) Neither		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) New drug therapies are being developed for cancer treatment of isolated organs. Research on several anesthetized animal models is being conducted prior to treatment of humans. Portable instrumentation is required for surgery on rats, and special catheters are required for liver isolation in pigs. This project involves the design of a portable physiological monitoring/surgical system for liver perfusion in rats, and design and fabrication of multilumen catheters for liver isolation in pigs.		

OBJECTIVES: To design a portable physiological monitoring system with sufficient working space to perform surgery on rats. A special low-volume oxygenator is also required for isolated liver perfusion. The following parameters will be monitored: perfused blood oxygen saturation, body temperature, ECG, and blood pressure.

PROGRESS: Several systems have been designed and built: (1) Two special double-lumen catheter connectors of different sizes were built for use in surgery on pigs. Because of the surgical difficulty in using this technique, a different and less demanding approach was tried. (2) A small surgical/monitoring table was built for use with rats. It included a pump (from a PancretecTM microinfusion pump), pressure transducers, a blood reservoir and circuit, a specially fabricated, hollow-fiber micropore tubing oxygenator, ECG probes, a thermistor for rectal temperature, and a pulse oximeter for blood oxygen saturation. Transducer amplifiers were acquired from BIOPAC Systems, Inc. (Goleta, California). Significant system improvement resulted from interfacing the transducers and amplifiers to a Macintosh computer for data acquisition and analysis. All system components were mounted on a movable table measuring 2 ft 6 in x 3 ft 5 in.

PROPOSED COURSE: All scheduled system modifications have been implemented. The system has been successfully used to provide liver perfusion during treatment with therapeutic agents. Based on the results of these animal studies, human trials will be conducted. No further action is required on this project.

In the future, there may be additional requirements for other organ-perfusion studies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10409-02 BEI									
PERIOD COVERED October 1, 1992 to September 30, 1993											
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Quasi-Elastic Light Scattering in Multiply Scattering Media											
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 40%;">Joseph M. Schmitt, Ph.D.</td> <td style="width: 30%;">Staff Fellow</td> <td style="width: 30%;">ACES, BEIP, NCRR</td> </tr> <tr> <td>Robert F. Bonner, Ph. D.</td> <td>Biophysicist</td> <td>EEES, BEIP, NCRR</td> </tr> </table>			Joseph M. Schmitt, Ph.D.	Staff Fellow	ACES, BEIP, NCRR	Robert F. Bonner, Ph. D.	Biophysicist	EEES, BEIP, NCRR			
Joseph M. Schmitt, Ph.D.	Staff Fellow	ACES, BEIP, NCRR									
Robert F. Bonner, Ph. D.	Biophysicist	EEES, BEIP, NCRR									
COOPERATING UNITS (if any) DCRT (R. Nossal, A. Gandjbakhche)											
LAB/BRANCH Biomedical Engineering and Instrumentation Program											
SECTION Applied Clinical Engineering Section											
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, Maryland 20892											
TOTAL STAFF YEARS: 0.1	PROFESSIONAL: 0.1	OTHER: 0.0									
CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">_ (a) Human subjects</td> <td style="width: 33%;">_ (b) Human tissues</td> <td style="width: 33%;">X (c) Neither</td> </tr> <tr> <td>_ (a1) Minors</td> <td></td> <td></td> </tr> <tr> <td>_ (a2) Interviews</td> <td></td> <td></td> </tr> </table>			_ (a) Human subjects	_ (b) Human tissues	X (c) Neither	_ (a1) Minors			_ (a2) Interviews		
_ (a) Human subjects	_ (b) Human tissues	X (c) Neither									
_ (a1) Minors											
_ (a2) Interviews											
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) In earlier studies we have shown that the pathlengths of photons propagating in dense suspensions of particles undergoing Brownian motion can be probed using quasi-elastic light scattering (QELS) techniques. Using similar techniques, we are now attempting to induce motions in a solid biological material to measure parameters of its cellular constituents.											

OBJECTIVES: To probe mechanical properties of biological tissues by using quasi-elastic light scattering (QELS) techniques.

METHODS EMPLOYED: The time-autocorrelation function of the backscattered intensity detected at the surface of a medium containing moving scatterers contains information about the size of the scatterers and the viscoelastic properties of the medium. For tenuous liquid media containing particles undergoing Brownian motion, theories have been developed to derive this information from the shape of the autocorrelation function measured at different scattering angles.

Because most biological tissues of interest are gel-like rather than liquid in their natural state, conventional QELS techniques cannot be applied to samples *in situ*. To overcome this limitation, we are investigating new QELS methods based on inducing motion in biological samples for determining physical properties of their constituents.

One simple method of providing a reproducible perturbation, developed by Dr. Nossal several years ago, employs a cuvette that is made to oscillate at several of its natural resonant modes by striking it with a metal rod. The intensity-correlation function of the scattered light is recorded while the sample is illuminated with a helium-neon laser beam.

The approach that we are now investigating is based on the use of magnetic beads bound to the sample. The beads are excited with an oscillating magnetic field from a coil that applies forces to the sample, and the intensity-correlation function of a laser beam scattered from the beads is recorded. It is possible to induce a variety of different motions, including sinusoidal and random motions, by simply changing the waveform of the coil's excitation voltage.

MAJOR FINDINGS: The feasibility of the magnetic excitation method has been demonstrated in samples of low viscosity. However, because the present apparatus applies insufficient force, the displacements induced in viscous samples are too small to produce measurable optical-phase modulation. This limitation hinders our ability to measure intensity-correlation functions in viscous gels.

SIGNIFICANCE: The viscoelastic properties of biological tissues depend on the structure and adhesion of their constituent cellular elements. The ability to assess these properties dynamically in small biological samples would facilitate scientific studies aimed at understanding the biochemical mechanisms of cell adhesion.

PROPOSED COURSE: The forces that are applied to the magnetic beads attached to the samples need to be increased. We plan to investigate ways of increasing the effective magnetic cross-section of the beads and/or the magnetic-field gradient applied to the beads.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10410-02 BEI
PERIOD COVERED October 1, 1992 to September 30, 1993		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) MRI Muscle Dynamometer		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Stephen B. Leighton, Sc.D. Mechanical Engineer MES, BEIP, NCRR Thomas R. Clem, Sr., BSEE Electrical Engineer EEES, BEIP, NCRR		
COOPERATING UNITS (if any) IR CE, NHLBI (T. Ryschon)		
LAB/BRANCH Biomedical Engineering and Instrumentation Program		
SECTION Mechanical Engineering Section		
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, Maryland 20892		
TOTAL STAFF YEARS: 1.3	PROFESSIONAL: 0.8	OTHER: 0.5
CHECK APPROPRIATE BOX(ES) X ☐ (a) Human subjects ☐ (b) Human tissues (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) It is useful to be able to make <i>in vivo</i> metabolic measurements (e.g., of muscle phosphocreatinine) with magnetic resonance spectroscopy while subjects are exercising specific muscle groups on a dynamometer. It is the purpose of the present work to design and construct a programmable wrist- and ankle-joint dynamometer for use in the magnetic environment of the NIH 4-Tesla MRI instrument.		

OBJECTIVES: Human studies of muscle energy metabolism during exercise have traditionally relied on chemical analysis of muscle samples obtained immediately following a trial of muscular work. Such tissue samples are acquired through a percutaneous approach to an easily accessible muscle mass, and must be limited in number to avoid functional disruption of the muscle. In addition, ethical considerations have precluded using this technique to study children's muscles. ^{31}P -NMR spectroscopy offers a noninvasive alternative to muscle biopsy for the measurement of high-energy phosphate concentration (ATP, Pi, ADP, PCr). In addition to being noninvasive, measurements can be acquired during exercise, and several parameters can be measured simultaneously, giving greater insight into the energetic processes involved in muscle contraction. Meaningful studies of muscular work require careful control and measurement of muscle tension. Bicycle ergometers, treadmills, and kinetic dynamometers are commonly used in the exercise physiology laboratory, but cannot be used in the NMR experiment for several reasons. The high-strength (4-Tesla) magnetic field required for NMR measurements precludes the use of any ferrometallic devices within 10 to 12 feet of the magnet. NMR measurements require the muscle to be placed in the center of the magnetic field, which is found midway inside the cylindrical bore of the magnet (5 meters in length and 70 cm in diameter). Thus, the dimensions and composition of any ergometer for use in NMR studies of humans must be unique, and cannot be satisfied by commercially available devices. An ergometer has been designed and constructed by the BEIP to meet these critical specifications. The ergometer will allow use with children, since this group of humans has not been adequately studied. The first implementation of the ergometer will be to study the high-energy phosphate concentration of contracting calf muscle in children at different stages of maturity and at different levels of physical conditioning. For this study, a pedal will be driven by the main ergometer, allowing precise control of the resistance to ankle flexion and extension, and precise measurement of the force produced in opposing this resistance. In subsequent studies, an arm module will be implemented to study the energetics of flexion and extension of the wrist. The ergometer will provide state-of-the-art, unprecedented control of the exercise being investigated.

We intend to create a system that is able to subject the ankle or wrist joints to programmed torques, position functions, or velocities. Magnitudes of the parameters will be adjustable to either adult or pediatric subjects. Torques will either resist or abet muscle motion, allowing both eccentric and concentric work to be studied. All parts of the system within the MRI magnet will be nonmagnetic, in order to avoid any interference with the spectroscopic measurements. All system controls and all logging of data will be handled by a remote personal computer.

METHODS EMPLOYED: A DC servo motor system is mounted on a cart positioned on the axis of the MRI coil, about 3 meters from the end of the coil. At this location, the magnetic field is weak

enough that the servo system is still functional. Likewise, the iron and working fields of the servo system are not expected to affect the operation of the MRI instrument. The output of the motor is impedance-matched to the load inertia with a 16:1 gearhead, and converted to nearly linear motion with a simple crank. A 5-meter-long rigid thin-wall tube structure acts as a push-pull link to connect the motor crank to the patient pedal within the magnet. The tube structure is aluminum, up to the magnet and fiberglass-reinforced epoxy composite within the magnet coil. An identical but stationary tube connects the motor base (cart) to the patient pallet so that reaction forces do not disturb the magnet or move the motor cart. A "panic button" and quick-release system allow the subject to be removed from the magnet on demand at any time.

Ankle-joint torque is monitored with a unique fiber-optic torque sensor, since electronic signals would be contaminated by artifacts from the motion in the magnetic field. A composite torque arm that connects the push-pull tube to the pedal shaft is engineered to have a specific deflection characteristic appropriate for this study. A non-loaded arm is mounted on the same shaft and thus moves with the loaded arm, except for the deflections of the latter created by any applied torque (analogous to a common torque wrench). A multifiber optical bundle terminates at the unloaded arm and faces a reflecting surface across the gap on the loaded arm. Increasing torque increases the gap and decreases the amount of light reflected from the light-delivery fibers in the bundle back to the light-receiving fibers in the same bundle. Electronic transduction of this signal is carried out well away from the MRI field.

The servo loop is closed by a commercial digital controller with a 1-KHz bandwidth, which accepts incremental encoder signals from the motor and sends commands to a 10-KHz-bandwidth switched-mode amplifier operating in a closed-velocity loop with a linear tachometer (also mounted on the motor). The controller accepts position commands downloaded from a personal computer (PC). The PC carries out data-logging tasks and overall system control. The controller may also be put in a torque mode, in which it compares the torque signal from the fiber-optic sensor to a desired torque waveform.

MAJOR FINDINGS: The device has been constructed and is being tested. So far, initial system specifications have been met.

SIGNIFICANCE: This apparatus will permit new studies of *in vivo* human muscle metabolism during specific exercise regimens, for elucidating new information on muscle mechanics and biochemistry.

PROPOSED COURSE: The tests will be finished and the apparatus installed in the 4-Tesla MRI instrument.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10412-01 BEI									
PERIOD COVERED October 1, 1992 to September 30, 1993											
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Electronic Stethoscope											
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 40%;">Walter S. Friauf, MEE</td> <td style="width: 40%;">Chief</td> <td style="width: 20%;">EEES, BEIP, NCRR</td> </tr> <tr> <td>Thomas R. Clem, Sr., BSEE</td> <td>Electrical Engineer</td> <td>EEES, BEIP, NCRR</td> </tr> </table>			Walter S. Friauf, MEE	Chief	EEES, BEIP, NCRR	Thomas R. Clem, Sr., BSEE	Electrical Engineer	EEES, BEIP, NCRR			
Walter S. Friauf, MEE	Chief	EEES, BEIP, NCRR									
Thomas R. Clem, Sr., BSEE	Electrical Engineer	EEES, BEIP, NCRR									
COOPERATING UNITS (if any) ROB, DCT, NCI (A. Russo)											
LAB/BRANCH Biomedical Engineering and Instrumentation Program											
SECTION Electrical and Electronic Engineering Section											
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, MD 20892											
TOTAL STAFF YEARS: 0.3	PROFESSIONAL: 0.3	OTHER: 0.0									
CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">_ (a) Human subjects</td> <td style="width: 33%;">_ (b) Human tissues</td> <td style="width: 33%;">X (c) Neither</td> </tr> <tr> <td colspan="3">_ (a1) Minors</td> </tr> <tr> <td colspan="3">_ (a2) Interviews</td> </tr> </table>			_ (a) Human subjects	_ (b) Human tissues	X (c) Neither	_ (a1) Minors			_ (a2) Interviews		
_ (a) Human subjects	_ (b) Human tissues	X (c) Neither									
_ (a1) Minors											
_ (a2) Interviews											
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Countless electronic stethoscopes have been developed during the past fifty years, but none has achieved any significant success. However, recent developments in small liquid crystal displays (LCDs) and advances in digital signal-processing components have rekindled interest in the possibility of developing a lightweight, portable, battery-powered instrument that would provide a visual output instead of (or in addition to) the usual audio output. This innovation could be particularly valuable to older physicians with hearing problems, as well as in facilitating the interpretation of the output signal. Direct visual display of the audio signal is not useful; rather, something more like an ECG signal, but having a greatly different spectrum and character, is desired.											

METHODS EMPLOYED: Audio signals for a variety of heart conditions were transcribed onto magnetic tape for subsequent playback and processing. Several simple processing functions were explored with a digital-processing oscilloscope, but no sequence of relatively simple functions was found that provided a display that physicians liked. Consequently, the signals were loaded into a personal computer (PC) and Gabor-transformed with a commercial software package. This approach shows considerable promise, but the transformation is far too slow to be useful for a stethoscope.

PROPOSED COURSE: We plan to continue the evaluation of signal-processing functions. If a fully satisfactory combination can be found, we will implement it in custom hardware with a liquid crystal display.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10413-01 BEI						
PERIOD COVERED October 1, 1992 to September 30, 1993								
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Female Rhesus Monkey Reproductive Physiology and Behavior Study								
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">Thomas J. Pohida, MSEE</td> <td style="width: 33%;">Electronic Engineer</td> <td style="width: 33%;">EEES, BEIP, NCRR</td> </tr> <tr> <td>Walter S. Friauf, MEE</td> <td>Chief</td> <td>EEES, BEIP, NCRR</td> </tr> </table>			Thomas J. Pohida, MSEE	Electronic Engineer	EEES, BEIP, NCRR	Walter S. Friauf, MEE	Chief	EEES, BEIP, NCRR
Thomas J. Pohida, MSEE	Electronic Engineer	EEES, BEIP, NCRR						
Walter S. Friauf, MEE	Chief	EEES, BEIP, NCRR						
COOPERATING UNITS (if any) LCE, NICHD (E. Phoebus)								
LAB/BRANCH Biomedical Engineering and Instrumentation Program								
SECTION Electrical and Electronic Engineering Section								
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, MD 20892								
TOTAL STAFF YEARS: 0.4	PROFESSIONAL: 0.2	OTHER: 0.2						
CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;"> ☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews </td> <td style="width: 33%;"> ☐ (b) Human tissues </td> <td style="width: 33%;"> ☒ (c) Neither </td> </tr> </table>			☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews	☐ (b) Human tissues	☒ (c) Neither			
☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews	☐ (b) Human tissues	☒ (c) Neither						
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.) Dr. Phoebus has a 7-channel commercial telemetry system, which he plans to implant in monkeys in order to monitor several physiological parameters. He wants to use a channel originally intended for EEG signals to monitor intrauterine pressure, utilizing a transducer he has on hand. An interface will be required between the pressure transducer and the FM telemetry system. Switchable battery power, also to be implanted, is necessary to provide an adequate system-life time after implantation. During system operation, general system support will be necessary and receiver data-processing hardware will need to be developed.								

OBJECTIVES: To design and construct six complete systems. The system design must provide high sensitivity, consume a minimal amount of power, be of minimal physical size, and sustain long-term implantation.

METHODS EMPLOYED: The system will primarily consist of two packages. The first package will include the commercial FM transmitter, the pressure transducer, and custom interface circuitry. EEES personnel designed the interface circuitry, which FM-modulates the pressure signal prior to input into the EEG channel. The design consumes less than 1 mA and is constructed with surface-mount components to maintain minimal size. The second package includes batteries and IR switches, which can be used to turn the system power off when data collection is not desired.

SIGNIFICANCE: The proposed telemetry system will acquire data that will be used to establish a biomedical animal model of female anorgasmia, which will enable Dr. Phoebus to study the effects on sperm migration, developmental factors in anorgasmia, and social organization.

PROPOSED COURSE: To complete construction of six systems, which includes the electrical assembly and the potting of the electronics in epoxy. (Testing will be performed prior to implantation.) To provide system support during data collection. To design an FM discriminator to extract pressure data from the 7-channel demodulator output.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10414-01 BEI
PERIOD COVERED October 1, 1992 to September 30, 1993		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) C-13 MRS Studies of Metabolic Paths and Conversion of Ibuprofen Enantiomers		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Ching-Nien Chen, Ph.D. Physical Scientist OD, BEIP, NCRR		
COOPERATING UNITS (if any) Rhode Island University (C-S. Chen); Howard University (P. C. Wang)		
LAB/BRANCH Biomedical Engineering and Instrumentation Program		
SECTION In Vivo NMR Research Center		
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, Maryland 20892		
TOTAL STAFF YEARS: 0.3	PROFESSIONAL: 0.3	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews ☐ (b) Human tissues ☒ (c) Neither		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Ibuprofen is an anti-inflammatory drug. This activity is based on its ability to inhibit the function of cyclo-oxygenase. It exists in two enantiomeric forms because of an asymmetric center alpha to the carbonyl group. In vitro studies have shown that ibuprofen is only active in the S-form enantiomer; conversion of the inactive R- to the active S-form in vivo has been postulated and supported by in vitro evidence. The study of ibuprofen metabolism has been accomplished mainly in vitro by studying pure compounds, or by sacrificing animals (rats) at certain time intervals after administering the drug and then monitoring the appropriate components, either in the plasma of total blood or by cellular extraction from the liver. The latter method is invasive and time-consuming, and can introduce unwanted errors. The purpose of our study is to verify the configuration conversion process of ibuprofen in vivo and to study its reaction mechanism and kinetics by using whole rats in an NMR imaging instrument. The ibuprofen will be labeled at the C2 position with 13-C and 2-H, and its 13-C NMR spectra variation will be followed from localized regions (preferably the liver) of the rat.		

METHODS EMPLOYED: We propose to study the uptake and conversion of ibuprofen in rat liver by following the NMR C-13 and/or H-2 signals at the asymmetric carbon center, C2. The R- and S-ibuprofen enantiomers (with and without simultaneous deuteration of the alpha carbon) and the R-form of the ibuprofen-CoA thioester intermediate can be synthesized. R- and S-[2-13C, 2-2H]-ibuprofen will be administered separately into the animal, and the time course of the C-13 and/or H-2 signals for each form will be observed. A superconducting magnet operating at 4.7T will be used. A surface coil or solenoidal coils around the rat will be used as the RF transmitter/receiver.

MAJOR FINDINGS: We have performed the above experiment on five rats, and the results so far indicate that the conversion can be followed by the proposed method. We observed the occurrence and disappearance of the thioester peak from the intermediate--the first evidence of an *in vivo* direct observation of drug configuration conversion known to us. A poster of the preliminary report was presented at the 5th International Symposium and Workshop of the Society of Chinese Bioscientists in America, June 15-20, 1993, in Baltimore, Maryland. A communication of the study is in preparation.

PROPOSED COURSE: Because of the low sensitivity of the appropriate signals, the method has to be revised so that we can quantify the data more accurately and study the kinetics. We are looking into improvements in both the hardware (e.g., the probe) and the NMR techniques (e.g., NOE).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER 201 RR 10415-01 BEI
PERIOD COVERED October 1, 1992 to September 30, 1993		
TITLE OF PROJECT (#0 characters or less. Title must fit on one line between the borders.) Automated Production of (18F)Fluorodopa		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Stephen B. Leighton, Sc.D. Mechanical Engineer MES, BEIP, NCRR Thomas R. Clem, Sr., BSEE Electrical Engineer EEES, BEIP, NCRR		
COOPERATING UNITS (if any) PET Imaging Section, NM, C C (W. Eckelman, M. Channing, P. Plascjak, H. R. Adams)		
LAB/BRANCH Biomedical Engineering and Instrumentation Program		
SECTION Mechanical Engineering Section		
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, MD 20892		
TOTAL STAFF YEARS: 0.3	PROFESSIONAL: 0.2	OTHER: 0.1
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews ☐ (b) Human tissues ☒ (c) Neither		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) An automatic system for synthesizing (18F)fluorodopa (and potentially other radiopharmaceuticals) is being developed. It is expected that this system will reduce operator exposure to ionizing radiation, improve product yields, and shorten production cycles. The automatic system is an improvement of an existing manual system that runs in a radioactive hot cell associated with the NIH cyclotron. The starting point of the reaction is (18F)F2 produced by the cyclotron. Steps include column absorbtions and elutions, vacuum evaporation and HPLC. The new system will be able to run unattended under computer control after initial loading of reagent reservoirs.		

OBJECTIVES: 1) To provide reproducible yields and product from a radiopharmaceutical synthesis. 2) To reduce total operator exposure to radiation. 3) To provide a compact apparatus such that two or three units might fit in a single hot cell. 4) To shorten synthesis time. 5) To provide a system that may be expanded to other syntheses. 6) To provide a system of moderate cost.

METHODS EMPLOYED: The existing chemical synthesis steps will be retained. The existing mechanical rotational evaporation apparatus will be replaced with a much smaller gas swirling evaporator with no moving parts and a solid-state heater. A compact steam generator will be used for *in situ* sterilization. Compact scintillation detectors will be used to monitor radiation levels throughout the process, and thus to compute chemical concentration levels. A peak detection algorithm will be developed for automatic endpoint determination following the final HPLC step. All components will be packaged in a compact manner consistent with ease of operator filling, cleaning, and maintenance.

MAJOR FINDINGS: The compact gas swirling evaporator has been designed and built, and so far has performed successfully in tests. This component lends itself to a flash evaporation method that may help reduce the total process time. Calculations and tests have shown that the compact steam generator should be able to provide adequate sterilization to the most remote parts of the system. The compact scintillation detectors and prototype shielding elements have been constructed. The heater and temperature monitoring and control elements have been built and are being assembled.

PROPOSED COURSE: The heater, controller, and swirl/evaporator will be combined and tested. The computer controller will be connected with the scintillation detectors and other control elements, and the entire system will be tested. As soon as the prototype system functions satisfactorily, it will be packaged to allow multiple units to fit in one hot cell.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10416-01 BEI
PERIOD COVERED October 1, 1992 to September 30, 1993		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Mathematical Modeling of Calcium Waves in Astroglia		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Bradley J. Roth, Ph.D. Senior Staff Fellow MES, BEIP, NCRR		
COOPERATING UNITS (if any) LMGD, NICHD (J. Russell)		
LAB/BRANCH Biomedical Engineering and Instrumentation Program		
SECTION Mechanical Engineering Section		
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, MD 20892		
TOTAL STAFF YEARS: 0.2	PROFESSIONAL: 0.2	OTHER: 0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews ☐ (b) Human tissues ☒ (c) Neither		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Calcium-dependent fluorescent dyes have been used to observe calcium waves in astroglia. Mathematical models exist that describe the behavior of these calcium waves in time, but their combined temporal/spatial behavior is unexplored. The goal of this project is to develop a model, similar to reaction/diffusion models for other excitable media, that is consistent with the experimental data. This model will be examined using computer simulations and emphasizing qualitative predictions that can be tested experimentally.		

OBJECTIVES: To increase our understanding of calcium waves in astroglia, and to provide a mathematical model that can be used to describe these waves.

SIGNIFICANCE: The function of glial cells in the nervous system is not known, but they may play a role in the slow responses of the nervous system. Astroglia can support waves of increased intracellular calcium concentration that propagate through the cell, which may provide one mechanism for signalling and communication in glial cells.

MAJOR FINDINGS: The calcium waves are generated by the release and subsequent uptake of calcium by the endoplasmic reticulum (ER). If the cell is modeled as having a heterogeneous distribution of ER, then the response of the cell to agonists is to induce oscillations of calcium with different frequencies at different locations within the cell. These oscillations are coupled by diffusion of calcium (or diffusion of some second messenger, such as IP3). The system can be modeled to a first approximation as a set of weakly coupled, nonlinear oscillators. This model explains some of the phenomena observed experimentally, such as wave collision and annihilation.

PROPOSED COURSE: A model will be developed for calcium wave propagation in glial cells. The kinetics of calcium release and uptake will be described using various published models. The significance on nonhomogeneities on wave propagation will be examined.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10417-01 BEI
PERIOD COVERED October 1, 1992 to September 30, 1993		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Gradient Coil Design for a Low-Frequency ESR Imaging System		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Bradley J. Roth, Ph.D. Senior Staff Fellow MES, BEIP, NCRR Seth Goldstein, Sc.D. Chief MES, BEIP, NCRR		
COOPERATING UNITS (if any) ROB, DCT, NCI (J. Bourg)		
LAB/BRANCH Biomedical Engineering and Instrumentation Program		
SECTION Mechanical Engineering Section		
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, MD 20892		
TOTAL STAFF YEARS: 0.25	PROFESSIONAL: 0.25	OTHER: 0
CHECK APPROPRIATE BOX(ES) _(a) Human _(b) Human X (c) Neither subjects tissues _(a1) Minors _(a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The Radiation Oncology Branch (ROB) of NCI is currently developing an electron spin resonance (ESR) imaging system. ESR spectroscopy systems usually operate at frequencies of about 10 GHz; however, in order to get sufficient tissue penetration to perform imaging, the ROB's system will work at a considerably lower frequency (e.g., 250 MHz). The line widths of typical ESR spectra are such that larger magnetic field gradients are needed for ESR imaging than for nuclear magnetic resonance imaging. The goal of this project is twofold: (1) to design a gradient coil system capable of producing magnetic field gradients on the order of 100 G/cm, to be used in the ROB's ESR imaging system; and (2) to assist the ROB in exploring the trade-offs between spatial resolution, tissue penetration, signal-to-noise ratio, and difficulty of construction that occur when modifying existing hardware and concepts from NMR imaging and ESR spectroscopy so that they apply to ESR imaging.		

OBJECTIVES: To design magnetic field gradient coils for use in a low-frequency electron spin resonance imaging system.

SIGNIFICANCE: ESR imaging may be useful for studying the spatial distribution of target molecules or endogenous paramagnetic molecules in animals. Because of the short relaxation times for electron spin systems, it may be possible to perform ESR imaging more rapidly than NMR imaging.

MAJOR FINDINGS: Because of the broad line width of electron spin resonance (ESR) spectra, very large magnetic field gradients are required (on the order of 100 G/cm) in order to obtain spatial resolution on the order of a millimeter from an ESR imaging system. The production of such field gradients requires large currents through coils wound with hundreds of turns of wire. These currents produce significant Joule heating, so that the coil must be water-cooled. An initial design of such a gradient coil system has been developed. It consists of three coil sets to produce magnetic field gradients in three perpendicular directions (dB_z/dx , dB_z/dy , and dB_z/dz), and one coil set to produce a uniform magnetic field in a single direction (B_z). The dB_z/dx and dB_z/dy coils have a Golay saddle coil design, the dB_z/dz coil has a Maxwell pair design, and the B_z coil has a Helmholtz pair design. Each coil set will be wound on a cylindrical former, so the entire system will consist of coils wound on four concentric formers, each with a different diameter. Each coil will have approximately 10 ohms of resistance, and will dissipate about 2.2 kw of power when carrying a current of 15 amps. The current will be generated by high-power switching amplifiers, so that arbitrary current wave forms (up to approximately 1 kHz) can be generated easily.

PROPOSED COURSE: The final design of the gradient coil system will be completed and the system will be built.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10418-01 BEI
PERIOD COVERED October 1, 1992 to September 30, 1993		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Transport of Macromolecules		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Cynthia Sung, Ph.D. Senior Staff Fellow CHES, BEIP, NCRR Robert L. Dedrick, Ph.D. Chief CHES, BEIP, NCRR		
COOPERATING UNITS (if any) SNB, NINDS (R. J. Youle, K. M. Muraszko, D. W. Laske); LMP, NCI (W. W. van Osdol, J. N. Weinstein)		
LAB/BRANCH Biomedical Engineering and Instrumentation Program		
SECTION Chemical Engineering Section		
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, MD 20892		
TOTAL STAFF YEARS: 1.2	PROFESSIONAL: 1.1	OTHER: 0.1
CHECK APPROPRIATE BOX(ES) X ☐ (a) Human subjects X ☐ (b) Human tissues (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Experimental and theoretical models have been developed to describe the in vivo transport of macromolecules such as protein toxins, immunotoxins, and monoclonal antibodies (MAbs). These have led to quantitative measurements of influx and efflux rates, as well as binding parameters, of these compounds in solid tumors. Quantitative autoradiography has been employed to assess the spatial distribution of intravenously administered immunotoxins in solid tumors. Two quantitative measures were devised that permit comparisons of the spatial distributions among immunotoxins that vary in molecular weight and degree of binding. The spatial heterogeneity observed in these experiments supports the use of spatially distributed models to describe macromolecular transport. We have developed such models for two-step protocols that use MAbs to achieve targeted delivery of a radionuclide. The theoretical results are being compared to experimental data obtained in a lung metastasis model. An immunotoxin injected directly into the cerebrospinal fluid has been evaluated for treatment of leptomeningeal neoplasia in a Phase I trial. A pharmacokinetic analysis of immunotoxin transport in the CSF has been performed and compared to preclinical experiments in rats and monkeys. These studies provide a basis for interspecies comparisons that will be applicable to future studies of CSF injection of macromolecules.		

RELEVANT BEIP PROJECTS: 86-128, 89-114, 92-122.

OBJECTIVES: To quantitatively characterize the transport properties of macromolecules by means of experimental and theoretical models, for the purpose of obtaining a clearer understanding of the factors that influence the distribution of these compounds in target and nontarget organs.

METHODS EMPLOYED: (1) An experimental system of human tumors grown in the flank of an athymic mouse was used to study the transport and binding characteristics of diphtheria toxin (DT), immunotoxins, and monoclonal antibodies (MAbs) in the tumors. A physiologically based, compartmental pharmacokinetic model was used to determine tumor and normal tissue capillary permeability to these macromolecules, lymphatic loss, and apparent antigen concentration. Model-fitted values of antigen concentration were consistently lower than values calculated from *in vitro* and *ex vivo* measurements--a possible indication that the distribution of macromolecules within the tumor tissue is heterogeneous. This discrepancy motivated autoradiographic studies to examine the spatial distribution of macromolecules in the tumors. Quantitative analyses were performed to calculate the fraction of the tumor's cross-sectional area that had attained a threshold concentration, and the kurtosis (a statistical measure of the dispersion in a distribution). The immunotoxin was distributed in punctate spots, and large portions of the tumor received little or no exposure to the immunotoxin. A 50 percent reduction in the molecular weight of the immunotoxin by use of a fragment of the MAb did not produce a significantly increased degree of homogeneity. Binding of the immunotoxin to the cells appeared to be a significant factor contributing to the heterogeneity. Another factor may be poor perfusion of capillary beds in the tumor. Literature reports indicate that angiotensin II opens up microvascular beds in the tumor; its use could potentially increase the surface area greatly for macromolecular transport into the tumor. We have undertaken experiments to study the effect of angiotensin II on tumor uptake and distribution of immunotoxins.

(2) Chemical engineering theories on diffusion and reaction were applied to describe two-step imaging or treatment protocols. These protocols involve injection of a modified MAb capable of binding both to tumor-associated antigen and to a second component that carries a radionuclide or drug. The second component is injected subsequently, after the MAb has distributed throughout the target tumors and been largely cleared from the plasma and normal tissues. The theoretical model includes the whole-body pharmacokinetics, transcapillary transport into normal tissue interstitium surrounding the nodule, binding of MAb to antigen, binding of the second component to the MAb, diffusion into a prevascular tumor nodule, and lymphatic outflow from the tissue space. Many of the model parameters were obtained or estimated from published reports or from our own experimental data. We have examined two protocols, one in which the first component is MAb

conjugated to streptavidin and the second component is radiolabeled biotin, and the other in which the first component is biotinylated MAb and the second component is radiolabeled streptavidin. Theoretical simulations of the latter protocol are being compared with experimental results obtained from metastatic lung tumors in the guinea pig.

(3) For the treatment of leptomeningeal neoplasia, drugs can be administered directly into the cerebrospinal fluid (CSF). A model-independent pharmacokinetic analysis has been completed of data from a Phase I clinical trial, in which patients received an injection of immunotoxin into the ventricular CSF space. Results were compared to preclinical studies in rats and monkeys. The results support the concept that pharmacokinetic data obtained in preclinical studies, namely the area under the concentration curve at the LD₁₀ for a species-matched immunotoxin, correlate with the maximally tolerated dose in humans.

SIGNIFICANCE: The models permit the testing of modifications of the experimental or clinical procedures to anticipate those that will have the greatest impact. Hence, utilization of experimental animals is reduced, and a more rational basis for optimization of the procedures is obtained. Interspecies correlations permit the design of more efficient clinical trials by better selection of the entry dose and the dose-escalation scheme.

PROPOSED COURSE: To complete experimental and theoretical work on the transport of immunotoxins under angiotensin II treatment. To continue modeling of the two-step protocols and correlating results with experimental findings. To extend the methods described herein to the study of the transport of peptides.

PUBLICATIONS: Sung C, Dedrick RL, Hall WA, Johnson PA, Youle RJ. The spatial distribution of immunotoxins in solid tumors: assessment by quantitative autoradiography. *Cancer Res* 1993;53:2092-9.

van Osdol WW, Sung C, Dedrick RL, Weinstein JN. A distributed pharmacokinetic model of two-step imaging and treatment protocols: application to streptavidin-conjugated monoclonal antibodies and radiolabeled biotin. *J Nucl Med* 1993 (in press).

Muraszko K, Sung C, Walbridge S, Greenfield L, Dedrick R, Oldfield EH, Youle RJ. Pharmacokinetics and toxicology of immunotoxins administered into the subarachnoid space in nonhuman primates and rodents. *Cancer Res* (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10419-01 BEI
PERIOD COVERED October 1, 1992 to September 30, 1993		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Aluminum in Alzheimer's Disease		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Carol R. Swyt, M.S. Physical Scientist OD, BEIP, NCRR		
COOPERATING UNITS (if any) LN, NIA (Q. Smith, Q. Deng, D. Brady); CAC, NIST (G. Gillen)		
LAB/BRANCH Biomedical Engineering and Instrumentation Program		
SECTION Electron Beam Imaging and Microspectroscopy Group		
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, Maryland 20892		
TOTAL STAFF YEARS: 1.0	PROFESSIONAL: 1.0	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews ☒ (b) Human tissues ☐ (c) Neither		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The role of aluminum in the etiology of Alzheimer's disease remains controversial because of the contradictory results of the many measurements of Alzheimer's brain specimens by methods including electron-probe x-ray microanalysis (EPMA), proton-induced x-ray emission (PIXE), secondary ion mass spectrometry (SIMS), and laser microprobe mass analysis (LAMMA). For many of these measurements, fixatives and stains were relied upon to facilitate the unambiguous identification of plaques and neurofibrillary tangles (NFTs), the indicators of the disease. However, the chemical processes of fixing and staining compromise the distributions and concentrations of aluminum and other elements of interest. Measurements on unfixed, unstained sections in which elemental distributions are assumed to be preserved are questioned because the identification of the structures being measured is difficult. A methodology which permits the unambiguous identification of plaques and NFTs, does not alter the elemental distributions, and provides detectability and rapid imaging of the aluminum distribution at concentrations in the ppm range is being developed. The methodology utilizes thioflavin-stained sections for observation by fluorescent LM, and freeze-dried cryosections for observation by electron and backscattered-electron imaging (SEI, BSI) and quantitative measurement by electron-induced x-ray microanalysis and SIMS.		

METHODS EMPLOYED: Thioflavin staining enables observation of NFTs and plaques by fluorescent-light microscopy. A modification of the Guntern thioflavin-staining protocol (Guntern et al., *Experientia* 48:8-10, 1992) is being developed in order to reduce tissue damage, but still allow verification that the CA1 layer of a specimen is rich in plaques and NFTs. Specimens are obtained from Alzheimer's brain hemispheres frozen at autopsy and maintained at -80°C. Sections are cut on a cryomicrotome, and one section from a series is air-dried, stained with thioflavin, and photographed in the LM. The CA1 layer and other tissue features are noted on the photographs. Based on the notable features, the CA1 layer in an adjacent unstained and freeze-dried section is identified by SEI in the EPMA (JEOL JXA-8600). Structures within the CA1 layer having the typical morphology of the pyramidal cell are identified by SEI and BSI, or structures with atomic numbers higher than the matrix average are detected by BSI. Digital maps of secondary and/or backscattered electrons from fields a few millimeters down to a few micrometers square are acquired and stored as digital images (modified Adobe Photoshop 2.0.1, Macintosh Quadra 900). The elemental composition of these structures is analyzed in the EPMA by energy-dispersive and wavelength-dispersive x-ray microanalysis (EDS, WDS). Two-dimensional digital elemental maps of fields in the order of 100 micrometers by 100 micrometers are then obtained by SIMS (CAMECA ims 4f) with less than the ppm detectibility for aluminum. The section remaining after SIMS mapping is again analysed by EPMA and then stained with thioflavin for observation in the LM. SEI and SIMS permit observation of surface characteristics, though in SIMS data is obtained as the surface layer is sputtered away to any desired depth. BSI and staining reveal characteristics of the entire thickness of a section. Correlation of LM, EPMA, and SIMS images permits identification of areas of significant elemental composition.

SIGNIFICANCE: The contradictory results of the many measurements of aluminum in Alzheimer's brain specimens leaves the role of the element in the disease process in question. It is uncertain if the extreme variation in results has been due to the preparation procedures or to real differences among samples measured. A methodology that enables the identification of plaques and NFTs, maintains the elemental distributions, and permits measurement of the concentration and distribution of aluminum at the ppm or ppb level would contribute to eliminating that uncertainty and to determining the significance of aluminum in the Alzheimer's disease process.

PROPOSED COURSE: A protocol that permits unambiguous identification of structures and meaningful quantitative measurement of the elemental compositions will be developed. Comparisons will be made of the morphology and composition of sections from 3µm to 20µm thick, dried by various procedures. Results of quantitative measurements will be correlated to patient history and pathology, as revealed by LM.

PUBLICATIONS: Gillen G, Swyt CR, Deng Q, Brady D, Smith Q. Methods for localization and imaging of trace element distributions in human brain neurons by secondary ion mass spectrometry. In: Nihei Y, ed. Proceedings of the Ninth International Conference on Secondary Ion Mass Spectrometry. Tokyo: John Wiley & Sons (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10420-01 BEI
PERIOD COVERED October 1, 1992 to September 30, 1993		
TITLE OF PROJECT (#0 characters or less. Title must fit on one line between the borders.) Thymidylate Synthase Regulation		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Paul F. Morrison, Ph.D. Physical Scientist CHES, BEIP, NCRR		
COOPERATING UNITS (If any) NCI-NMOB (C. Allegra, E. Chu, D. Voeller)		
LAB/BRANCH Biomedical Engineering and Instrumentation Program		
SECTION Chemical Engineering Section		
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, MD 20892		
TOTAL STAFF YEARS: 0.5	PROFESSIONAL: 0.5	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews ☐ (b) Human tissues ☒ (c) Neither		
SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.) The biosynthesis of thymidylate synthase (TS) has recently been found to be down-regulated by binding of the protein to its own mRNA, and this regulation is involved in the development by cancer cells of resistance to several antimetabolites targeted at this enzyme. We are presently engaged in the physical characterization and quantification of this process. NCI-NMOB has observed that both the TS/mRNA binding and TS activity are strongly affected by the presence of mercaptoethanol (ME) and other reducing agents. We have hypothesized that both binding and activity are dependent on a reversible sulfhydryl switch, probably involving one or more cysteines near the active pocket of the enzyme. To test this hypothesis, we have constructed a kinetic model of such a process and examined it for its ability to account for experimental observation. The model allows for the binding of TS to two different mRNA locations chemically cleaved by T1-RNase during assay: the reversible redox switch, an unknown amount of active TS introduced into the assay before addition of ME; and an ordered mechanism for synthesis of thymidine from uridylate and methylenetetrahydrofolate. We estimated a binding constant of 0.76 nM, a redox equilibrium constant of 4×10^{-9}, and an initial TS active fraction of 1.1%. Most importantly, we found no significant difference between parameters estimated either from a fitting of combined binding and enzyme data sets or individual fittings, suggesting that the same reducing site is involved in both binding and active pocket locales. Furthermore, the calculated enzyme activity has been found to be in very close agreement with TS activity obtained from <i>lactobacillus casei</i>.		

RELEVANT BEIP PROJECTS: 93-104, Thymidylate Synthase Regulation.

OBJECTIVES: To physically characterize and quantify the regulation of thymidylate synthase production controlled by the binding of that protein to its own messenger. To explore this regulation in order to identify agents or protocols useful in circumventing some of the drug resistance associated with up-regulation of this system.

METHODS EMPLOYED: A biochemical kinetic model has been developed to describe the binding and redox reactions. The redox reaction between enzymatically active and inactive TS, 2-mercaptoethanol, and 2-hydroxyethylthiol has been described algebraically as a Gibbs equilibrium, while the binding between active TS and the two mRNA binding fragments has been described differentially. Combined with appropriate conservation of mass and stoichiometry relations, this formalism allows parameter estimation similar to Scatchard analysis but yields intrinsic rather than apparent binding constants and properly accounts for system nonlinearities. Regression methods based on the method of steepest descents were used to estimate parameters and their asymptotic variance-covariance matrix. Experimental determination of the concentrations of TS/mRNA^{TS} complexes involved binding of ¹⁴C-labelled messenger to TS followed by digestion of the unbound mRNA segments by T1 nuclease and subsequent resolution of the complexes on polyacrylamide gels with quantitation by autoradiography.

MAJOR FINDINGS: The major finding was that parameter estimates obtained from fittings to combined binding and enzyme activity data did not differ significantly from those obtained from individual fits to the binding and activity data, thus supporting the hypothesis that a common reduced site is involved in both protein interactions. Furthermore, we found less than a threefold difference in binding affinities between the two mRNA binding fragments. The set of values for the (single) binding affinity (K), the redox equilibrium constant (K*), the fraction of active TS initially present in the solutions undergoing binding to mRNA (f), and the reaction rate constant for production of thymidylate from uridylate and methylenetetrahydrofolate (k) was {K, K*, f, k} = {0.76 x exp(±0.161) nM, 4.1 x 10⁻⁹ x exp(±0.269), 0.0098 x exp(±0.178), 9.1 x exp(±0.265) μM/min} when obtained from fits to combined binding and activity data. They were {K, K*, f} = {0.92 x exp(±0.637) nM, 6.7 x 10⁻⁹ x exp(±1.50), 0.012 x exp(±0.608)} when obtained from fits to binding data alone, and {K*, f, k} = {1.3 x 10⁻¹¹ x exp(±2.92), 0.056 x exp(±1.19), 33 x exp(±1.25) μM/min} when derived from fits to enzymatic activity alone. Parameters are very consistent between fits of combined data and fits of binding data only. Mean differences are larger between combined and enzymatic data fits but do not reach significance because of the large log normal variances associated with the enzymatic fits. In addition, the combined fit parameters are further substantiated by predictions

of thymidylate synthase activity that are in excellent agreement with published reports of the dependence of *lactobacillus casei* TS activity on ME concentration.

SIGNIFICANCE: The finding that both binding and enzyme activity are under the control of a reversible, reducible site in TS (most likely cysteine 50 and/or 146) joins the observations of others that a free protein sulfhydryl group (or groups) is required in order for bacteriophage coat protein, aminoacyl-tRNA synthetase, and iron-responsive element-binding protein to bind to their respective mRNAs. Hence, a reversible sulfhydryl switch may be indicated as a general mechanism of protein synthesis regulation.

PROPOSED COURSE: The binding between TS and its messenger is thought to occur between a surface site on TS (under the control of the redox state of a cysteine, but not necessarily at the same physical locus) and a stem-loop structure of mRNA^{TS}. Computations have predicted such stem-loop formation, but the structures have neither been observed directly nor shown to exist stably at 37°C. Hence, efforts are presently underway to produce TS-mRNA^{TS}-antiTS complexes of sufficient molecular weight to allow their visualization by electron microscopy. In addition, it is anticipated that either UV absorbance or circular dichroism may be employed to investigate the thermal stability of the stem loops and thus confirm their existence at physiological temperature. A manuscript entitled "The Effect of Reducing Reagents on Binding of Thymidylate Synthase Protein to Thymidylate Synthase Messenger RNA" is in preparation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10421-01 BEI
PERIOD COVERED October 1, 1992 to September 30, 1993		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Luminescence Assay for Pharmaceutical Screening		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Alec Eidsath, Ph.D. Staff Fellow MES, BEIP, NCRR		
COOPERATING UNITS (if any) LCB, NIMH (D. Button, M. Brownstein)		
LAB/BRANCH Biomedical Engineering and Instrumentation Program		
SECTION Mechanical Engineering Section		
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, Maryland 20892		
TOTAL STAFF YEARS: 0.2	PROFESSIONAL: 0.2	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects X ☐ (b) Human tissues (c) Neither ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The gene for the photoprotein aequorin has been incorporated into several mammalian cell lines. Because the luminescence of this protein reflects the local Ca^{++} concentration, it is very useful for assaying cellular Ca^{++} signals that are activated, or blocked, by drugs of potential therapeutic value. A major experimental difficulty lies in the very low level of light that is emitted, since only one photon is liberated by each molecule of aequorin, and we have calculated that there are only 20,000 molecules per cell. We plan to modify a photon-counting instrument that we built to measure luminescence from frog oocytes. The photon count will be used as an assay to determine the activity of drugs that influence cell Ca^{++} metabolism. Large numbers of chemicals will be screened for activity, so the instrument will be automated as much as possible. We have the difficult job of injecting small quantities (20 to 50 microliters) of fluids and cell suspensions. If the preliminary results are positive, we will consider purchasing an autosampler to go along with the instrument. Another of the difficulties in the design is the "sticky" character of many of the drugs, even to stainless steel. Since even small residues from prior experiments could affect the luminescence, all of the reusable pieces and tubing will have to be made of Teflon TM .		

OBJECTIVES: To measure the calcium signal caused by receptor binding in a mammalian cell.

METHODS EMPLOYED: A cooled photomultiplier with very low dark counts is hooked up to the counter of a multipurpose A/D board. The experiment is controlled and data are stored using LabView on the Macintosh.

SIGNIFICANCE: This instrument will be a great aid in discovering new agonists and in cloning receptors.

PROPOSED COURSE: The initial testing of the instrument showed that we have the sensitivity to measure the calcium surge in the cell population after the addition of a known agonist (e.g., a histamine). We found that too rapid an injection of a control (i.e., the buffer) could lead to a false-positive response. Great care, therefore, has to be taken to avoid high shear stresses when introducing fluids.

Future modifications for the instrument will include:

A. Allowing it to perform measurements on more than one type of cell sample (e.g., adherent cells grown on the surface of test tubes and suspensions of cells dispensed into the instrument's sample chamber).

B. Interfacing it to an automated fluid-handling system to increase the throughput of the experiment. Here, we will have to take great care with such things as the pH and pCO_2 of the cell media so that the luminescence of the cells is not affected prior to delivery to the sample chamber.

C. Exploring the shear-stress dependence of the response by using a parallel-plate flow cell and cells adhering to a cover slip.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10422-01 BEI
PERIOD COVERED October 1, 1992 to September 30, 1993		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <u>Biomedical Applications of Optical Low-Coherence Reflectometry</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Joseph M. Schmitt, Ph.D. Staff Fellow ACES, BEIP, NCRR Robert F. Bonner, Ph.D. Biophysicist EEES, BEIP, NCRR		
COOPERATING UNITS (if any) IR CE, NHLBI (A. Knüttel); IR LCB, NHLBI (J. Knutson); PSL, DCRT (A. Gandjbakhche)		
LAB/BRANCH Biomedical Engineering and Instrumentation Program		
SECTION Applied Clinical Engineering Section		
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, Maryland 20892		
TOTAL STAFF YEARS: 1.0	PROFESSIONAL: 0.9	OTHER: 0.1
CHECK APPROPRIATE BOX(ES) _ (a) Human _ (b) Human X (c) Neither subjects tissues _ (a1) Minors (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Optical-coherence reflectometry (OCDR) was first introduced as a method for high-resolution probing of reflections from components of fiber-optic systems. We have been exploring the application of this technique to imaging and characterization of biological tissues. Several prototype systems have been built in the last year to identify the fundamental problems involved in the development of practical imaging systems and potential uses of the technology in clinical diagnosis.		

OBJECTIVES: To develop new imaging techniques based on low-coherence ("white-light") interferometry, which would enable high-resolution imaging of a superficial layer of living tissue. An image of structures as small as a few microns, embedded as deep as 1 to 2 mm in optically dense tissues, can potentially be captured in real time and displayed in a cross-sectional format similar to that of an ultrasonic B-scan image.

METHODS EMPLOYED: An OCDR system is based on an interferometer illuminated by a continuous-wave broadband light source. The mirror in one of the arms of the interferometer is replaced by the sample, and the mirror in the other (reference) arm is translated to vary the optical-path difference. Fringes are produced only when the optical paths of the light reflected from the sample and from the reference mirror differ by less than the coherence length of the light source. When light-emitting diodes (which have short coherence lengths) are employed as sources, pathlength differences as small as a few microns can be resolved. By recording the fringe magnitude versus the displacement of the reference mirror, we can obtain a profile of the coherent optical power backscattered by the sample over a range of optical distances.

Several low-coherence reflectometry systems based on these principles have been constructed in our lab to measure backscattered signals from living tissues and scattering models with tissue-like properties. To analyze the backscattered intensity-versus-depth profiles obtained from the samples, we have applied a first-order scattering model that accounts for the effects of the focal spot size and light-collection angle on the heterodyne efficiency. The experimental data are compared with theoretical predictions to identify the variables that are important in the design of OCDR systems for probing biological tissues.

Using the best of our prototype systems, we have obtained 1-D profiles of light backscattered from human skin tissue to a depth of about 750 μm . With this system, structures separated by about 25 μm could be resolved. Two-dimensional images of excised bone specimens were also reconstructed from profiles recorded by the laboratory reflectometer, by translating the specimen laterally in small increments.

Most recently, we have been developing depth-profiling reflectometers that obviate the moving mirror in the reference arm of the interferometer by using a detector array to record fringe patterns produced by diverging reference and sample beams. In addition to their simpler construction, these stationary reflectometers potentially enable faster data acquisition, a requirement for measuring profiles on living tissues.

MAJOR FINDINGS: Our theoretical studies have shown the importance of the focal spot size and angular field-of-view of the illumination/collection optics on the efficiency of coherent-light

detection from biological tissues. If the focal spot over which a reflectometer collects light is made large enough, a simple attenuation model can be used to relate the slope and offset of the measured reflectance curves. In samples containing particles that scatter light in the forward direction over very narrow angles (a few degrees), the contribution of the multiple scattered light to the coherent backscattered light collected from locations deep in the sample may be substantial.

In our experimental studies, we demonstrated that optically dense suspensions can be probed to depths of several hundred microns. From the measured reflectance profiles, their backscattering coefficients and total attenuation coefficients could be assessed rapidly and nondestructively. Reflectance profiles from living skin showing anatomical details of the uppermost skin layers were also obtained, although they were more difficult to measure. In some applications, two-dimensional images similar to those made on an excised bone specimen in this study may prove to be most useful for visualizing extended lesions. However, to make imaging by low-coherence optical reflectometry practical for clinical application, improvements in the signal-to-noise ratio and the data acquisition rate of currently available reflectometers are required.

SIGNIFICANCE: Imaging and spectroscopy using low-coherence interferometry may potentially enable noninvasive diagnosis or characterization of superficial lesions in any tissue accessible from the surface of the body. Assessment of retinal damage, early detection of skin melanomas, and measurement of burn depth are a few of the specific applications that have been suggested. Also, a variety of applications in biomedical research can be imagined, such as the quantification of the extent of atherosclerotic lesions in pathological studies.

PROPOSED COURSE: The major challenges involved in the development of imaging systems for routine application in research and clinical medicine are of both a technical and a conceptual nature. Because the coherent-light intensities backscattered from tissues are very weak, extraordinary efforts need to be made to maximize the dynamic range and the signal-to-noise ratio of the interferometer that forms the heart of the reflectometer. Before such instrumentation can be used effectively, however, a better theoretical understanding of the scattering of coherent light in tissues needs to be gained. We plan to work toward solving these problems by designing more sensitive and faster instruments, while studying at the same time how coherent optical waves with different characteristics propagate in a tissue medium.

PUBLICATIONS: Schmitt JM, Knüttel A, Bonner RF. Measurement of optical properties of biological tissues by low-coherence reflectometry. Appl Opt 1993 (in press).

Knüttel A, Schmitt JM. Stationary depth-profiling reflectometer based on low-coherence interferometry. Opt Commun 1993 (in press).

Schmitt JM, Knüttel A, Gandjbakhche A, Bonner RF. Optical characterization of dense tissues using low-coherence interferometry. In: Proceedings of the SPIE. 1993 (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10423-01 BEI
PERIOD COVERED October 1, 1992 to September 30, 1993		
TITLE OF PROJECT (#0 characters or less. Title must fit on one line between the borders.) "The Head and Heart of Chaos" Workshop		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) James R. Ellis, Ph.D. Electronics Engineer ACES, BEIP, NCRR		
COOPERATING UNITS (if any) CNP, NINDS (F. J. Nice); OD, DH, NHLBI (D. M. Robinson); DICBR, NIAAA (U. E. Ruttiman); NIMH; OMAR; Naval Research Laboratory (A. C. Ehrlich); Department of Neurosurgery, Children's Hospital (K. S. Madden); Synectics Corporation (A. E. R. Woodcock); L. Rosenbaum, Clinical Psychologist		
LAB/BRANCH Biomedical Engineering and Instrumentation Program		
SECTION Applied Clinical Engineering Section		
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, MD 20892		
TOTAL STAFF YEARS: 2.9	PROFESSIONAL: 1.9	OTHER: 1.0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews ☐ (b) Human tissues ☒ (c) Neither		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Some members of the Inter-Institute Chaos Council (IICC) determined that a forum was needed to exchange ideas concerning the behavior of nonlinear systems and their sometimes unpredictable behavior in biological and medical systems. They organized a workshop of interested contributors and potential new users to exchange ideas about problems and solutions. The workshop, which focused on neurophysiology and cardiology, was entitled "The Head and Heart of Chaos: Nonlinear Dynamics in Biological Systems." The first two resultant publications are an executive summary and an edited transcript of the proceedings of the workshop. They are designed to help make the material presented and discussed in the workshop readily available in written form.		

OBJECTIVES: (1) To clarify, for an eclectic audience, the state of the art of nonlinear sciences, deterministic chaos, and nonlinear dynamics as they apply to biomedical research, particularly with respect to cardiology and neurophysiology. (2) To show how the new nonlinear dynamical techniques resemble (and differ from) more established linear and nonlinear techniques for data analysis. (3) To indicate the accomplishments, problems, and critical considerations for distinguishing promising research from weak or erroneous research. (4) To suggest what types of research and/or data sets are most likely to provide the information that will clarify the value of nonlinear dynamical techniques in the near term.

METHODS EMPLOYED: The workshop, entitled "The Head and Heart of Chaos: Nonlinear Dynamics in Biological Systems," was held June 15 and 16, 1993, in the Masur Auditorium of the Clinical Center, National Institutes of Health, Bethesda, Maryland.

SIGNIFICANCE: The mathematics and physics needed to understand and apply results from chaos to biology and medicine are likely to remain difficult and uncomfortable for nonmathematicians. Similarly, mathematicians and others will find it difficult to learn about biological and medical applications. The workshop addressed these difficulties in a personal and intensive way. The executive summary represents the introductory remarks, the roster of participants, and abstracts of the presentations. The edited transcript provides a record of the proceedings.

MAJOR FINDINGS: There proved to be a significant demand for this type of forum. A workshop with the support of OMAR and three NIH institutes was organized. There was great interest and support from the extramural biological, medical, mathematics, and physics communities. Preregistration for the workshop was cut off at a point over 600 participants, due to the capacity of Masur Auditorium. Almost all of the registered speakers and discussants came, and the auditorium was filled to within three or four seats of capacity during the two-day workshop.

PROPOSED COURSE: The paper or hard copy transcript document has been finished and transmitted to personnel at the NHLBI, who have deposited it with the National Technical Information Service. Copies will be made available to the public on request.

The executive summary of the workshop has been published and distributed in hard copy form. An electronic or soft copy form has been made available for FTP browsing or downloading on the Internet.

PUBLICATIONS: Raub WF, DeLisi C, Madden KS. The head and heart of chaos: nonlinear dynamics in biological systems, executive report. Bethesda, MD: National Institutes of Health (OMAR), 1992.

Rosenbaum L, Ehrlich AC, Ellis JR, Nice FJ, Woodcock AER, eds. The head and heart of chaos: nonlinear dynamics in biological systems, edited proceedings. Springfield, VA: National Technical Information Service, 1993.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10424-01 BEI
PERIOD COVERED October 1, 1992 to September 30, 1993		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) NMR Center Workstations and NMR Center Internetwork (SPINet)		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Geoffrey Sobering, Ph.D. Senior Staff Fellow NMR, BEIP, NCRR Yoseph Shiferaw, Ph.D. Staff Fellow NMR, BEIP, NCRR		
COOPERATING UNITS (if any) None		
LAB/BRANCH Biomedical Engineering and Instrumentation Program		
SECTION In Vivo NMR Research Center		
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, MD 20892		
TOTAL STAFF YEARS: 0.6	PROFESSIONAL: 0.6	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews ☐ (b) Human tissues ☒ (c) Neither		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The NMR Center maintains a collection of UNIX-based workstations for use by the NMR Center user community. The demand for off-line processing is increasing dramatically. In the past year, Sun4 workstations have been added, along with 2 Sun file servers. A continued increase in demand for resources is anticipated, and additional workstations will be purchased as the need arises. These workstations are connected to the NMR Center network, which provides access to all the NMR instruments in the Center, as well as to the NIH backbone. Another responsibility is the design, installation, testing, performance monitoring, and maintenance of the NMR Center network (SPINet). The NMR Center contains an internetwork consisting of thick-ethernet, thin-ethernet and local-talk physical media. This internetwork uses the TCP/IP and Appletalk protocol suites. A variety of bridging and interconnection devices are used to interconnect the various physical and logical networks. As the NMR Center grows, the network must be adapted and modified to suit. It is anticipated that in the next year, the current single physical ethernet segment will be split into a number of logically connected but physically separated segments.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10425-01 BEI
PERIOD COVERED October 1, 1992 to September 30, 1993		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Omega System Maintenance, Protocol Development, and Operator Training		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Alan W. Olson, M.S. Electronics Engineer NMR, BEIP, NCRR		
COOPERATING UNITS (if any) None		
LAB/BRANCH Biomedical Engineering and Instrumentation Program		
SECTION In Vivo NMR Research Center		
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, MD 20892		
TOTAL STAFF YEARS: 0.7	PROFESSIONAL: 0.7	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) ☐ (a) Human subjects ☐ (a1) Minors ☐ (a2) Interviews ☐ (b) Human tissues ☒ (c) Neither		
SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.) The principal investigator performs periodic performance checks, routine component changes, and cryogen ordering and filling as needed for operation of the General Electric Omega 2-Tesla and 4.7-Tesla NMR systems, and assists Omega system operators as needed with protocol development and continuing training.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10426-01 BEI									
PERIOD COVERED October 1, 1992 to September 30, 1993											
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Apple Macintosh Computer Administration at the NIH In Vivo NMR Research Center											
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">Geoffrey Sobering, Ph.D.</td> <td style="width: 33%;">Senior Staff Fellow</td> <td style="width: 33%;">NMR, BEIP, NCRR</td> </tr> <tr> <td>Denise Sobering, B.A.</td> <td>Secretary</td> <td>NMR, BEIP, NCRR</td> </tr> </table>			Geoffrey Sobering, Ph.D.	Senior Staff Fellow	NMR, BEIP, NCRR	Denise Sobering, B.A.	Secretary	NMR, BEIP, NCRR			
Geoffrey Sobering, Ph.D.	Senior Staff Fellow	NMR, BEIP, NCRR									
Denise Sobering, B.A.	Secretary	NMR, BEIP, NCRR									
COOPERATING UNITS (if any) None											
LAB/BRANCH Biomedical Engineering and Instrumentation Program											
SECTION In Vivo NMR Research Center											
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, MD 20892											
TOTAL STAFF YEARS: 0.4	PROFESSIONAL: 0.1	OTHER: 0.3									
CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">_ (a) Human subjects</td> <td style="width: 33%;">_ (b) Human tissues</td> <td style="width: 33%;">X (c) Neither</td> </tr> <tr> <td colspan="3">_ (a1) Minors</td> </tr> <tr> <td colspan="3">_ (a2) Interviews</td> </tr> </table>			_ (a) Human subjects	_ (b) Human tissues	X (c) Neither	_ (a1) Minors			_ (a2) Interviews		
_ (a) Human subjects	_ (b) Human tissues	X (c) Neither									
_ (a1) Minors											
_ (a2) Interviews											
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The NMR Center maintains a collection of Macintosh computers and peripherals used in the daily operation of the Center, as well as for various research tasks. It is necessary to upgrade software and maintain the instruments on a regular basis. The demand for the use of Macintosh computers for off-line processing of data from all of the magnets in the Center is increasing. It has become necessary to dedicate a computer for transfer of data from the magnet computers to other computers for data processing. In addition, the Macintosh computers have proven useful in the preparation of images for submission to journals and societies.											

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10427-01 BEI
PERIOD COVERED October 1, 1992 to September 30, 1993		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Development of <i>In Vivo</i> Proton NMR Spectroscopy and Spectroscopic Imaging		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Chrit Moonen, Ph.D. Manager NMR, BEIP, NCRR Geoffrey Sobering, Ph.D. Senior Staff Fellow NMR, BEIP, NCRR		
COOPERATING UNITS (if any) CBDB, NIMH; LDRR, OD (J. Frank, J. Duyn); Johns Hopkins University (P. van Zijl); Pittsburgh NMR (J. Gillen)		
LAB/BRANCH Biomedical Engineering and Instrumentation Program		
SECTION In Vivo NMR Research Center		
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, MD 20892		
TOTAL STAFF YEARS: 0.3	PROFESSIONAL: 0.3	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) X ☐ (a) Human ☐ (b) Human (c) Neither subjects tissues ☐ (a1) Minors ☐ (a2) Interviews		
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.) The purpose of this project is the noninvasive evaluation of regional biochemistry.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10428-01 BEI															
PERIOD COVERED October 1, 1992 to September 30, 1993																	
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Development of the Use of Field Gradients in High-Resolution NMR Spectroscopy																	
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 40%;">Chrit Moonen, Ph.D.</td> <td style="width: 30%;">Manager</td> <td style="width: 30%;">NMR, BEIP, NCRR</td> </tr> <tr> <td>Geoffrey Sobering, Ph.D.</td> <td>Senior Staff Fellow</td> <td>NMR, BEIP, NCRR</td> </tr> <tr> <td>Daryl DesPres, B.S.</td> <td>Biologist</td> <td>NMR, BEIP, NCRR</td> </tr> <tr> <td>Guoying Liu, Ph.D.</td> <td>Visiting Fellow</td> <td>NMR, BEIP, NCRR</td> </tr> <tr> <td>Peter van Gelderen, Ph.D.</td> <td>Visiting Fellow</td> <td>NMR, BEIP, NCRR</td> </tr> </table>			Chrit Moonen, Ph.D.	Manager	NMR, BEIP, NCRR	Geoffrey Sobering, Ph.D.	Senior Staff Fellow	NMR, BEIP, NCRR	Daryl DesPres, B.S.	Biologist	NMR, BEIP, NCRR	Guoying Liu, Ph.D.	Visiting Fellow	NMR, BEIP, NCRR	Peter van Gelderen, Ph.D.	Visiting Fellow	NMR, BEIP, NCRR
Chrit Moonen, Ph.D.	Manager	NMR, BEIP, NCRR															
Geoffrey Sobering, Ph.D.	Senior Staff Fellow	NMR, BEIP, NCRR															
Daryl DesPres, B.S.	Biologist	NMR, BEIP, NCRR															
Guoying Liu, Ph.D.	Visiting Fellow	NMR, BEIP, NCRR															
Peter van Gelderen, Ph.D.	Visiting Fellow	NMR, BEIP, NCRR															
COOPERATING UNITS (if any) Johns Hopkins University (P. van Zijl)																	
LAB/BRANCH Biomedical Engineering and Instrumentation Program																	
SECTION In Vivo NMR Research Center																	
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, MD 20892																	
TOTAL STAFF YEARS: 0.9	PROFESSIONAL: 0.8	OTHER: 0.1															
CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">X ☐ (a) Human subjects</td> <td style="width: 33%;">☐ (b) Human tissues</td> <td style="width: 33%;">☐ (c) Neither</td> </tr> <tr> <td>☐ (a1) Minors</td> <td></td> <td></td> </tr> <tr> <td>☐ (a2) Interviews</td> <td></td> <td></td> </tr> </table>			X ☐ (a) Human subjects	☐ (b) Human tissues	☐ (c) Neither	☐ (a1) Minors			☐ (a2) Interviews								
X ☐ (a) Human subjects	☐ (b) Human tissues	☐ (c) Neither															
☐ (a1) Minors																	
☐ (a2) Interviews																	
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) In this project, the plan is to speed up multidimensional spectroscopy and to acquire new information with the help of self-shielded, high-performance field gradients.																	

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10429-01 BEI												
PERIOD COVERED October 1, 1992 to September 30, 1993														
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Development of Functional MRI Approaches														
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 40%;">Chrit Moonen, Ph.D.</td> <td style="width: 30%;">Manager</td> <td style="width: 30%;">NMR, BEIP, NCRR</td> </tr> <tr> <td>Geoffrey Sobering, Ph.D.</td> <td>Senior Staff Fellow</td> <td>NMR, BEIP, NCRR</td> </tr> <tr> <td>Guoying Liu, Ph.D.</td> <td>Visiting Fellow</td> <td>NMR, BEIP, NCRR</td> </tr> <tr> <td>Yoseph Shiferaw, Ph.D.</td> <td>Staff Fellow</td> <td>NMR, BEIP, NCRR</td> </tr> </table>			Chrit Moonen, Ph.D.	Manager	NMR, BEIP, NCRR	Geoffrey Sobering, Ph.D.	Senior Staff Fellow	NMR, BEIP, NCRR	Guoying Liu, Ph.D.	Visiting Fellow	NMR, BEIP, NCRR	Yoseph Shiferaw, Ph.D.	Staff Fellow	NMR, BEIP, NCRR
Chrit Moonen, Ph.D.	Manager	NMR, BEIP, NCRR												
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Guoying Liu, Ph.D.	Visiting Fellow	NMR, BEIP, NCRR												
Yoseph Shiferaw, Ph.D.	Staff Fellow	NMR, BEIP, NCRR												
COOPERATING UNITS (if any) Flow and Metabolism Laboratory, NIAAA (A. McLaughlin); Neuroimaging Laboratory, DIR, NINDS (G. DiChiro); CBDB, NIMH (D. Weinberger); LDRR, OD (J. Frank, J. Pekar)														
LAB/BRANCH Biomedical Engineering and Instrumentation Program														
SECTION In Vivo NMR Research Center														
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, MD 20892														
TOTAL STAFF YEARS: 0.6	PROFESSIONAL: 0.6	OTHER: 0.0												
CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">X ☒ (a) Human subjects</td> <td style="width: 33%;">☐ (b) Human tissues</td> <td style="width: 33%;">☐ (c) Neither</td> </tr> <tr> <td>☐ (a1) Minors</td> <td colspan="2"></td> </tr> <tr> <td>☐ (a2) Interviews</td> <td colspan="2"></td> </tr> </table>			X ☒ (a) Human subjects	☐ (b) Human tissues	☐ (c) Neither	☐ (a1) Minors			☐ (a2) Interviews					
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☐ (a1) Minors														
☐ (a2) Interviews														
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.) The purpose of this project is to acquire functional data in addition to the high-quality anatomical data currently obtained with MRI. The following functions are being studied: flow, perfusion, diffusion, oxygen consumption, and metabolism. Aspects under study are NMR pulse sequences, image processing, and relation to PET.														

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10430-01 BEI
PERIOD COVERED October 1, 1992 to September 30, 1993		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Functional MR Imaging		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation) Ronald L. Levin, Sc.D. Biomedical Engineer MES, BEIP, NCRR		
COOPERATING UNITS (if any) NIMH (J. Haxby); LNS, NIA (T. Zeffiro)		
LAB/BRANCH Biomedical Engineering and Instrumentation Program		
SECTION Mechanical Engineering Section		
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, Maryland 20892		
TOTAL STAFF YEARS: 0.2	PROFESSIONAL: 0.2	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) X (a) Human (b) Human (c) Neither subjects tissues (a1) Minors (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Over the past several years, it has become apparent that MR (magnetic resonance) can provide information other than of a purely anatomical or diagnostic nature. MR has been shown to be sensitive to physiological and pathological processes, particularly in the brain. These so-called "functional" MR techniques may one day rival existing PET (Positron Emission Tomography) techniques as the methods of choice for the noninvasive visualization and analysis of neural activity. As such, NIH is starting a large multi-ICD effort in the area of functional MR research. BEIP has been asked to play a supporting role in this effort.		

OBJECTIVES: To facilitate the use of magnetic resonance imaging and spectroscopy for the functional visualization of brain activity.

PROPOSED COURSE: (1) To develop MR-compatible test equipment, similar to that already available for PET-based functional research, for the stimulation of visual, auditory, and human motor control activity under well-controlled conditions using echo-planar functional MR scanning. (2) To use NIH's new **MEDx** image-processing package for the visualization and analysis of functional MR data. This goal includes the incorporation of advanced segmentation, registration, fusion, and rendering algorithms into **MEDx**, as well as the conversion of many heretofore PET-specific visualization and analysis routines (e.g., the SPM package from Hammersmith Hospital in London) into more robust routines that are capable of handling MR data.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 RR 10431-01 BEI															
PERIOD COVERED October 1, 1992 to September 30, 1993																	
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Development of <i>In Vivo</i> NMR Diffusion Spectroscopy and Imaging																	
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 40%;">Chrit Moonen, Ph.D.</td> <td style="width: 30%;">Manager</td> <td style="width: 30%;">NMR, BEIP, NCRR</td> </tr> <tr> <td>Geoffrey Sobering, Ph.D.</td> <td>Senior Staff Fellow</td> <td>NMR, BEIP, NCRR</td> </tr> <tr> <td>Peter van Gelderen, Ph.D.</td> <td>Visiting Fellow</td> <td>NMR, BEIP, NCRR</td> </tr> <tr> <td>Guoying Liu, Ph.D.</td> <td>Visiting Fellow</td> <td>NMR, BEIP, NCRR</td> </tr> <tr> <td>Daryl DesPres, B.S.</td> <td>Biologist</td> <td>NMR, BEIP, NCRR</td> </tr> </table>			Chrit Moonen, Ph.D.	Manager	NMR, BEIP, NCRR	Geoffrey Sobering, Ph.D.	Senior Staff Fellow	NMR, BEIP, NCRR	Peter van Gelderen, Ph.D.	Visiting Fellow	NMR, BEIP, NCRR	Guoying Liu, Ph.D.	Visiting Fellow	NMR, BEIP, NCRR	Daryl DesPres, B.S.	Biologist	NMR, BEIP, NCRR
Chrit Moonen, Ph.D.	Manager	NMR, BEIP, NCRR															
Geoffrey Sobering, Ph.D.	Senior Staff Fellow	NMR, BEIP, NCRR															
Peter van Gelderen, Ph.D.	Visiting Fellow	NMR, BEIP, NCRR															
Guoying Liu, Ph.D.	Visiting Fellow	NMR, BEIP, NCRR															
Daryl DesPres, B.S.	Biologist	NMR, BEIP, NCRR															
COOPERATING UNITS (if any) University of Delft, The Netherlands (T. Mehlkopf); Johns Hopkins University (P. van Zijl)																	
LAB/BRANCH Biomedical Engineering and Instrumentation Program																	
SECTION <i>In Vivo</i> NMR Research Center																	
INSTITUTE AND LOCATION NCRR, National Institutes of Health, Bethesda, MD 20892																	
TOTAL STAFF YEARS: 1.0	PROFESSIONAL: 0.9	OTHER: 0.1															
CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">_ (a) Human subjects</td> <td style="width: 33%;">_ (b) Human tissues</td> <td style="width: 33%;">X (c) Neither</td> </tr> <tr> <td colspan="3">_ (a1) Minors</td> </tr> <tr> <td colspan="3">_ (a2) Interviews</td> </tr> </table>			_ (a) Human subjects	_ (b) Human tissues	X (c) Neither	_ (a1) Minors			_ (a2) Interviews								
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_ (a1) Minors																	
_ (a2) Interviews																	
SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.) The purpose of this project is the study of (restricted) mobility of metabolites and water for a clarification of local physical chemical parameters, such as viscosity, membrane transport, and membrane permeability.																	



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